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EFFECTS EVOKED IN AN AXON BY THE ACTIVITY OF A CONTIGUOUS ONE*†

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(Received for publication June 3, 1941)

THERE IS no doubt that the activity of an element in the midst of a cell agglomeration can influence that of its neighbors, even when specialized contact surfaces for transmission, *i.e.* those loci traditionally known as synapses and which have been endowed with particular properties are lacking.

When the cell-units already are rhythmically active, their rhythm may synchronize more or less rigidly about that of one of the elements, which thus becomes the "pace-maker." Analysis of this is favorable in the agglomeration of cells of a nervous tissue, owing to the absence of mechanical factors which must enter into the synchronization of cardiac cells, of elementary muscular contractions (4) or of spermatozoa tails (60).

Amongst the phenomena of synchronization of nervous elements, that of cortical and ganglion cells (2, 3, 5, 5a, 59) are specially interesting. But although the vicinity interactions certainly play an important part in these phenomena, it is not always possible to eliminate that played by the specialized synapses. (See however experiments of Libet and Gerard, 51, 59). Synchronizations observed on isolated neural conductors do not suffer this difficulty.

Adrian (1) suggested that the synchronization of impulses in the isolated phrenic is possible because "an active fibre can cause a slight momentary increase in the stimulus to other fibres and that it can do so owing to the action current which it produces."

Certain conditions are required for the phenomenon to appear and be lasting. Arvanitaki and Fessard (19) showed that, for two spontaneous fibres of the crab nerve to interact, (i) their natural periods must be very close to one another, and (ii) the beats must be fairly well in phase. Also, the action potential of the pace-making element must be of sufficient amplitude. Thus, when several fibres with small action potentials and beating with similar rhythms do not synchronize, a large spike potential in a contiguous fibre may set them off together; but after a few beats in phase they again desynchronize (Arvanitaki, 10, p. 118, Fig. 63).

A rhythmically active element may progressively capture new quiescent

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* Owing to the present circumstances this paper, finished since May 1940, could not be published earlier.

† Correspondence concerning this manuscript was prevented by war developments. It has been rewritten and shortened with every effort to retain the author's interpretations and references. For any misconstructions of her intent, I must assume responsibility. R.W.G.

elements so that an ever larger and more spreading response follows the rhythm of the pace-making element; the recruitment phenomena observed on myelinated (49) and on crab nerve (Arvanitaki, 10, p. 118).

Finally, a real transmission has been observed. In a crab nerve a single impulse arriving at a differentiated point may introduce a rhythmic impulse discharge in the same fibre and in a contiguous one; a pseudoreflex effect (8, 9, 21). Jasper and Monnier (56) more directly proved transmission of excitation from an element in one crab nerve to another placed in contact. The transmission delays they record were very variable but averaged 28 msec. Such considerable delays are hardly consistent with the required speed of transmission in synchronization and recruitment phenomena. Lastly, the older experiments of Bethe, proving that reflex movements of the crab antenna could be procured after ablation of the cell bodies—recently confirmed (70) on the stellar ganglion of Loligo—should probably be interpreted as an axon-axon transmission between the pre- and postganglionic fibres at the level of the section.*

Such preparations involve many unknowns: the number of active fibres, the elements influenced, the position and condition of the contact surfaces, the relation of pickup to the critical locus, the amount of shunting, etc. Conditions for analysis are much more favorable with an axon to axon preparation of the isolated giant fibres of *Sepia*. With this the strict determinism of the transmission of excitation at the zone of contact, and intermediary mechanisms which determine graded subliminal effects, are brought to light.

The experimental axon-axon contact had been referred to as an "experimental axon-axon synapse" (15, 16). But the word "synapse," of established usage and designating certain anatomically differentiated loci with particular properties, should perhaps be avoided. The mechanisms of transmission are doubtless similar in the two cases but not identical; and the contiguity interactions deserve separate consideration, for they may be important in nervous physiology and pathology. We therefore propose the term "ephapse"† to designate the locus of contact or close vicinity of two active functional surfaces, whether this contact be experimental or brought about by natural means. It may be the locus of contact of two absolutely homomorphous surfaces, for instance an axon-axon or soma-soma contact of two nerve cells. It would therefore differ from the word synapse‡ whose meaning is narrower and designates surfaces of contact (whether axosomatic, axodendritic, or axomuscular) anatomically differentiated and functionally specialized for the transmission of the liminal excitations from one element to the following in an irreciprocal direction.

* Compare the experiments of Osterhout and Hill (65) and of Auger (20) showing transmission of excitation from one plant cell to another contiguous one or across a salt bridge.

† From εφαπτω, to touch on or to, to attach; hence εφαψις, the action of touching.

‡ From συναπτω, to touch or attach together, to contact; hence συναψις is the action of joining, of linking, the union.

METHODS

Two giant axons of *Sepia officinalis* (69) of equal diameter (200 to 300μ) and 3 to 4 cm. long were isolated as described (11). The normal and identical excitability and conductivity along both axons are then assured by spike potential tests. The first axon, A, is placed on 4 leads, *ab* for stimulation and *cd* for recording, the end resting on small paraffin supports. Axon B is placed in contact with axon A for a length of about 5 mm. (*gh*) and on leads *ij*, *i* being close to the contact zone (Fig. 1).

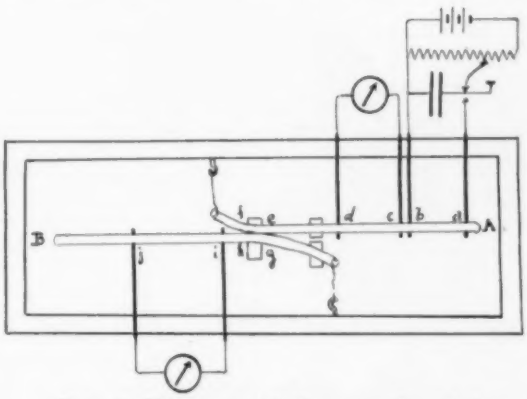

FIG. 1. Diagram of the experimental arrangement of the axon-axon preparation
A. afferent or preephaptic axon
B. effector or postephaptic axon
a, *b*, stimulation leads—*c*, *d* and *i*, *j* recording leads.
d and *i* can be moved and accurately adjusted.

Particular care was taken that the leads were well insulated to avoid leaks and the formation of secondary stimulation leads. The leads on which axon A rests were not supported by the same wall as those on which axon B rests. Leads were chlorided silver blades covered with threads soaked in seawater and fixed with sealing-wax into holes in the ebonite wall of the chamber. Condenser discharges were used for stimulation. Potentials were amplified with direct coupled amplifiers and the deflections of moving iron oscillographs (Dubois) recorded by means of a bromide paper camera. To record the potentials between leads *cd* and *ij*, two completely independent amplifiers with balanced input stages (63) were used.

EXPERIMENTAL RESULTS

We shall call the stimulated axon an *afferent* or *preephaptic axon*, and its impulse similarly. Axon B, subjected to the action of A, and its response, will be designated as *effector* or *postephaptic*. Two cases must be considered: (i) the postephaptic axon B initially at rest and (ii) this axon spontaneously active.

I. The postephaptic axon initially at rest

The afferent axon A is stimulated by a brief condenser discharge of subthreshold intensity. The local response, or prepotential, near cathode *b* is recorded in *cd* (Fig. 2). As the intensity of the stimulus is gradually increased, the height of the local response increases progressively but no deflection is recorded on fiber B from leads *ij* (Fig. 2, I). When, however, a minute increase of the stimulus brings it to threshold a spike is recorded in *cd* (Fig. 2, I'A) and activity is suddenly exhibited by leads *ij* (Fig. 2, I'B). A brief diphasic deflection *pn* (a passive electric "escape" from the afferent spike) at *ij* is followed by a local wave of negativity *l* which represents sub-liminal activity evoked in region *gh* upon arrival of the afferent spike. Reducing the stimulus again eliminates all trace of activity at *ij*.

The preephaptic spike thus behaves towards axon B as a sub-liminal electric stimulus. To increase the relative stimulating value of the pre-

ephaptic impulse on region *gh*, this portion of axon B is soaked in a Na enriched solution isotonic to sea-water or, better, in a mixture of 8 parts of sea-water to 2 parts of isotonic trisodium citrate solution. Axon B is then placed as before and axon A stimulated. No activity is recorded by leads *ij* as long as the stimulus remains sub-threshold to A (Fig. 2, II), but as soon as a propagated response appears in A (Fig. 2, II′), activity also appears in B. A propagated B spike, starting from the top of the local response evoked in *gh*, is recorded in *ij* and, as electrical escape, e, upon the negative after-potential of fiber A (Fig. 2, II′A, see also Fig. 3). Washing the junctional region with a few drops of sea-water causes the response of axon B to become again sub-liminal although the spike at *cd* remains (Fig. 3, III); and resensitizing the contact zone *gh* with a drop of citrate solution immediately reestablishes the propagated response of B (Fig. 3, IV). This reversal can be repeated several times with no displacement of the axons, and therefore with no change in electrical relations, and the results described are infallibly obtained on every proper preparation.

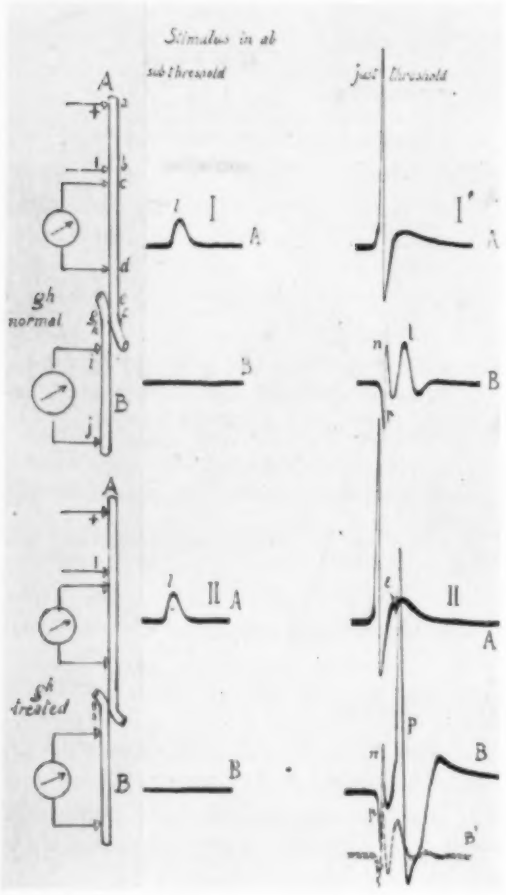

FIG. 2. Simultaneous records by means of two independent amplifiers of the responses of axons A and B, contiguous in *ef gh*, to a sub- or just-threshold stimulus applied at *ab*.

When the stimulus is sub-threshold and only a local response is recorded at *cd* (IA, IIA) no deflection appears at *ij*, whether region *gh* is normal (IB) or has been treated with citrate (IIB).

When the stimulus is just-threshold, a propagated spike recorded at *cd* (I′A, II′A) evokes a local sub-liminal response *l* (I′B) of the effector axon if region *gh* is normal and a liminal response P, if region *gh* has been treated (II′B).

These results can be summed up as follows: When the threshold of the postephatic axon is not sufficiently low, the incident spike potential evokes merely a local electric response in the contact zone, just as does a brief artificial electric stimulus of sub-threshold intensity.* When the threshold of the effector axon is lowered suffi-

* In a recent important paper Lorente de Nó (62), discussing his model of synaptic transmission, opened the quèstion (see p. 449) whether a local "active" response might not be implied besides the "passive" electrotonic propagation.

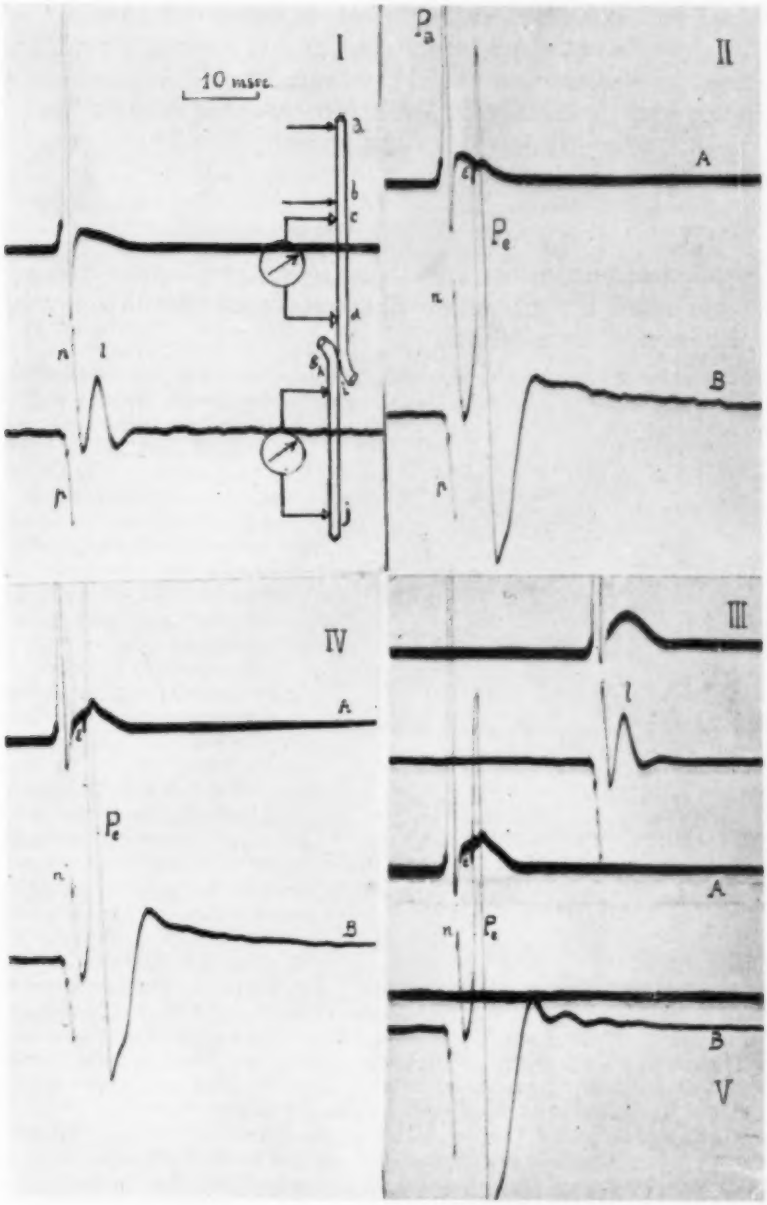

FIG. 3. Simultaneous records from the preephaptic axon, A (top curves) and the postephaptic axon, B (lower curves) of the responses evoked by a just-threshold electric stimulus applied at *ab*, as the region *gh* is altered:

I. *gh* normal. The reaction of axon B is only sub-liminal. After the diphasic effect of the afferent spike (see text), *pn*, recorded by electrode *i*, the prepotential *l* develops.

II. After citrate treatment. The reaction of axon B is liminal and an efferent spike P_e, is recorded at *ij*.

III. After washing. The reaction of axon B is again sub-liminal.

IV. Renewed citrate.

V. Third citrate treatment. The efferent spike P_e, is here followed by a local subliminal oscillatory response. Note that the transmission delay, *i.e.* the interval between arrival of the afferent spike at the contact region (indicated by the negative deflection *n*, see text) and the start of the efferent spike, is here 2 msec. shorter than in IV, B, and still shorter than in II, B.

ciently an afferent spike becomes a threshold stimulus and evokes the same response in the efferent axon as does a liminal electric stimulus; in both cases a spike starts at the top of the local response. This preparation of two isolated axons experimentally brought into contact is thus the prototype for transmission of excitation from one element to another, including subliminal as well as liminal effects.

The effective stimulus

In this preparation it is obvious that the external action currents due to the spike conducted by the active fibre represent the virtual stimulus to region *gh*, its immediate neighbor.

The effectiveness of the stimulus depends on the electric field created by this primary event in the exterior conductive media, particularly in region *gh*. An exact knowledge of the distribution of isopotential lines of this field and of their variation with the propagation of the spike along axon A would therefore be necessary here. It is on their characteristics that the variations of potential gradient to which the influenced segment is subjected will depend—variations which more directly determine the stimulating value of the afferent spike.

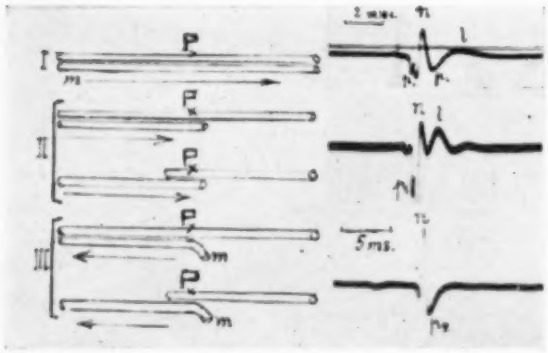

FIG. 4. Variations of the potential of an external point, P, as recorded by an interposed resting axon, when a spike in a contiguous active axon is propagated in the direction of the arrows (see text).

The electric field created by the action currents of an element in the conductive media that surround it has been considered from various viewpoints (26, 27, 28, 36, 45, 62, 68). Wilson, McLeod and Barker (68), after mathematical analysis, conclude that an action potential advancing in a linear element produces a potential change at any point in the surrounding conducting medium as would a similarly advancing dipole. Bishop (27) has reached similar conclusions as to the variations of potential at an exterior point, P, from consideration of the lines of force about a potential across the membrane of the conducting element. Relative to a distant point, M, the potential of P becomes: first relatively more positive as the spike approaches (phase p_1); then relatively negative (phase n), the maximum being reached when the excitation wave passes immediately under P; then again relatively positive (phase p_2), as the wave, having passed under P, moves away.

The conditions about point P may be less symmetrical. If, for example, the propagation stops in the conducting element immediately under P, the potential change at P will be restricted to the two first phases, p_1 and n. Conversely, if the wave starts under P, only the negative and second positive phases will appear (Fig. 4, III). And if P is far from the conductor only the positive phase may persist.

These three cases can be realized by leading from a resting axon the spike passing in a contiguous axon. The first is seen when axon A, having met the efferent axon, lies alongside it for some distance. The resulting triphasic wave (Fig. 4, I) lasts 1.5 to 2 msec., corresponding to the propagation time along A. Obviously, such an arrangement is not favorable to start a response of the efferent fiber. As a matter of fact, when this triphasic variation is followed by a prepotential wave, this wave is of negligible amplitude.

The second case is obtained when the spike in A is recorded from leads *ij* on axon B (Fig. 4, II); and the third when a spike started in region *gh* of axon B is recorded from electrodes *cd* on axon A. In this third case, the appearance of some or no negativity pre-

ceding the positivity depends on the distance of electrode *d*. Activity of B never evokes a response in A.

It is thus only in the second case, *i.e.* when external action currents due to the propagation of the afferent spike terminate in a more or less pronounced negativity, that subthreshold or threshold stimulation of the efferent fiber results. Actually the amplitude of the efferent response is a function of that of the negative phase, n, of the diffuse currents. Figure 5, for instance, reproduces the responses recorded from *ij* on axon B following threshold stimulation of axon A at *ab*, as region *gh* of B is progressively altered by citrate. Records I to III show the height of the prepotential response, *l*, increasing with the negative phase of the currents due to the afferent spike. Plotting as abscissae the heights of the negative phase of the "diffusion" wave and as ordinates the heights of the corresponding prepotential response gives the curve of Fig. 6. It is essentially the curve for electric stimulation of an isolated axon of the crab (52, 58) or of *Sepia* (12), when abscissa is the intensity of the stimulating current and ordinate the height of the prepotential. The negative portion of the external action currents thus represents the useful stimulating part for a second axon.

The visible evidence that citrate increases the height of the action currents of A which pass through B suggests that citrate diminishes the impedance of the membrane of the fiber on which it acts.

Since the negative phase of these currents tends to excite neighboring elements, the positive phases might similarly depress. Actually, in cases I and III the results are as if the final anodic positive wave were depressing the active response evoked by the cathodic stimulus. Katz and Schmitt (57) have recently shown a polyphasic change of excitability in a crab fiber when an impulse passes through an adjacent fiber. Their experimental conditions correspond to case I and in duration and configuration the excitability changes they describe correspond phase by phase to the curve of Fig. 4, I. The change of excitability and the change of external potential here considered are presumably concomitant phenomena.

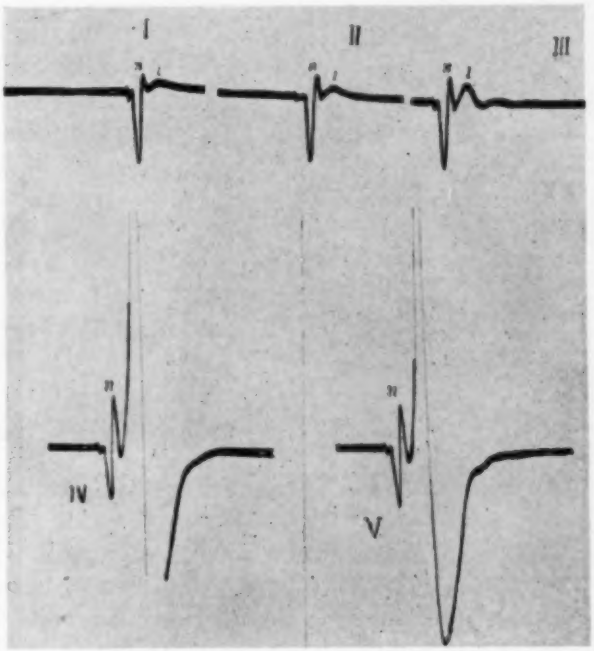

FIG. 5. Effects evoked by an afferent spike on the efferent axon B. Region *gh* is normal in I, and progressively citrated in II, III, and IV. The height of the negative phase, *n*, of apparent currents increases from I through IV. The local sub-liminal efferent action, *l*, increases at the same time from I to III and becomes liminal and propagated in IV and V.

Although not further discussed here, prepotentials and after-potentials of the afferent axon, as well as spike potentials, can produce detectable variations in the excitability of the efferent axon.

In short, the characteristics of the effective stimulus—duration, shape and amplitude—depend partly on the characteristics of the spike potential and on its propagation speed and partly on the distributed resistances and capacities of the external medium, as well as on the relative positions of the active and the influenced segments. And, as with any electric stimulus, the

changes in the influenced segment prior to its active response, *e.g.* its prepotential, are purely passive electric phenomena.

Delay of ephaptic transmission of the excitation

This delay represents the interval between the entry of the incident spike into the ephaptic region and the initiation of the postephaptic spike. It can be calculated precisely when axon A is long enough to permit the direct measurement of propagation velocity in it. Propagation time to the ephapse (3 msec. on the average) is then subtracted from the interval between the afferent spike recorded in *cd* and the efferent spike recorded in *ij* (under conditions of liminal transmission). This delay reaches a maximum value of 5 msec. The same value for the delay is obtained by taking the time of the negative wave in the potential field (leads *ij*) as the index of the arrival of the afferent spike at the ephapse.

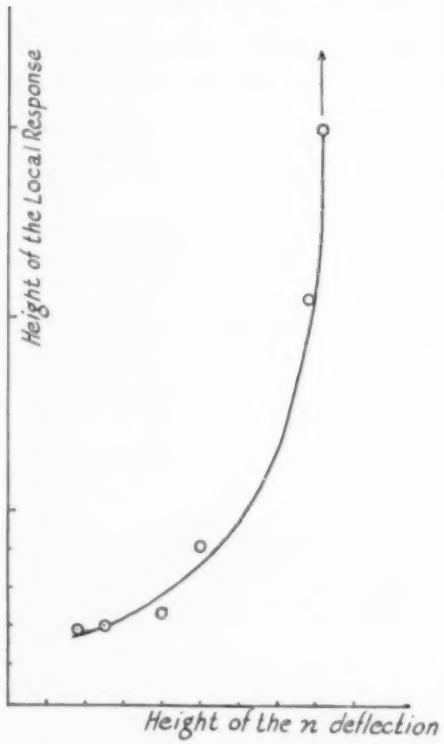

FIG. 6. Relation between the negative phase, *n*, due to afferent action currents and the height of the post-ephaptic local response, *l*. A spike starts on the prepotential response at the point marked by an arrow.

Two factors at least may contribute to this delay: (i) an interval before the postephaptic local response starts; and (ii) the time necessary for this response to reach its liminal level to set off the spike. The second factor is the more important and, as the excitability of axon B increases, and so the stimulating value of the incident spike, the duration of the ascending phase of the local potential decreases and the ephaptic delay with it. Variation of the ascending phase from 5 to 2 msec. (3.5 in Fig. 3, II, 2 in 3, V) is paralleled by similar variations of ephaptic delay. In myelinated axons, Blair and Erlanger (29) have shown a related fluctuation of latency of response to threshold induction shocks, over a 0.25 msec. range for most excitable axons, of 2.5 msec. for least excitable ones; as compared to a minimal latency of only 0.1 msec. with suprathreshold stimuli. The fluctuation is presumably here also in axon reactivity and in the development of the local response.

The spike potential of the preephaptic axon thus comports itself towards the postephaptic axon exactly as an artificial electric stimulus would have done. If the effector axon is relatively inexcitable, its response will be re-

stricted to the prepotential wave. If its excitability is such that the incident impulse should act as a liminal stimulus, the local response develops further and from the top of the prepotential wave, after time for its rise, a spike is propagated all along the fiber.

One particular case deserves further note. Under mild citrate action, the sub-threshold response of the treated region changes the aperiodic to the damped oscillatory type (12). This oscillatory response can be evoked not only by make or break of a constant current but also by a brief stimulus in the region of the cathode (Fig. F) or by a spike potential conducted along

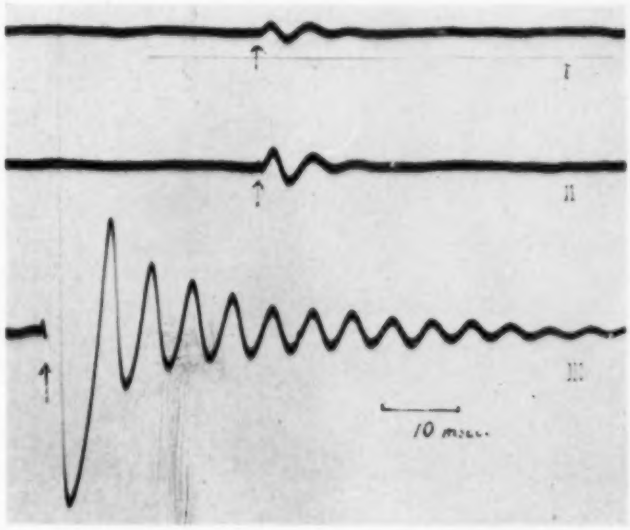

FIG. 7. Local oscillatory response evoked in a citrated axon region near the cathode of a brief sub-threshold condenser discharge. Three successive records show gradually increasing intensity of the stimulus marked by arrows. In III this is threshold and a spike starts on the first negative local wave.

the same axon to the citrated region (13). These same damped oscillatory reactions can be observed on the postephaptic axon B as a sub-liminal response to an incident impulse from A, after B has been repeatedly treated with citrate solution and washed with sea-water (Fig. 2, II′B′ and Fig. 3, III). After further citrate treatment of region *gh* of axon B, the arrival of an afferent spike, P_a, initiates an efferent spike, P_e, followed by a series of damped subliminal oscillations of the local potential (Fig. 3, V). P_e always starts at the top of the negative phase of the first sub-liminal undulation after the interval required for the local potential to reach its liminal value. If stimulation by an afferent spike is repeated several times a "facilitation" of the response results; the sub-liminal oscillations following the efferent spike increase in height until a rhythmic and sometimes prolonged discharge of spikes follows the first efferent spike. This may be called a postephaptic after discharge.

Thus, when the postephaptic axon is in the state to develop a sub-liminal oscillatory activity, it will respond to an incident impulse of subthreshold intensity with a series of regularly damped oscillations of its local potential, and to one of threshold intensity with an efferent spike starting at the top

of the first negative oscillation and followed by an after discharge that may be sub-liminal (damped oscillations of the local potential) or liminal (rhythmic impulse discharge). Again, the response to an incident impulse is exactly what it would have been to an electric stimulus.

II. The postephaptic axon is initially active

A. Sub-liminal oscillatory activity

Such oscillations can continue as long as several seconds, without initiating propagated spikes, and maintain a remarkably constant period, of the order of 7 to 10 msec. for the isolated axon of *Sepia* (12), and of 8 to 10 msec. for crab fibers (6). Such a sub-liminal activity often remains after a discharge of rhythmic impulses, spontaneous or evoked. Repeated treatment of the contact region *gh* of axon B with a sodium rich solution may finally lead to such a discharge following a single incident impulse from axon A. During such oscillatory activity of B, single afferent impulses set up in A (by condenser discharges) (Fig. 8) could be caused to reach B in different phases of its oscillations. The arrival of the afferent spike in the contact region *ef* is timed on record B by the negative phase of its escape (deviation *n*).

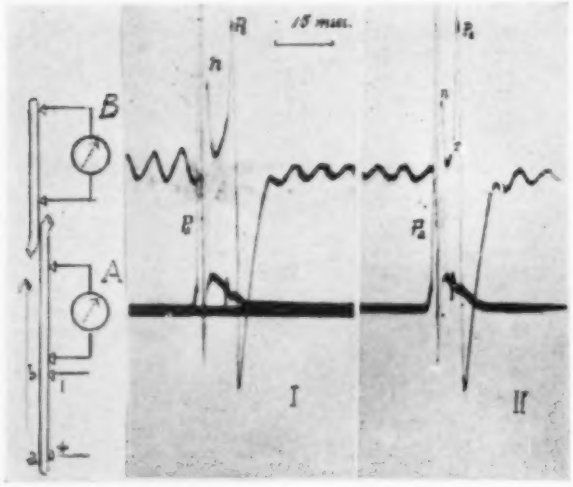

FIG. 8. Simultaneous records, from independent amplifiers of the responses of two axons continguous at *ef gh*, to an efferent spike evoked at *ab*. Axon B initially exhibited a sub-liminal oscillatory activity (upper record). Upon the arrival of the afferent spike P_a (lower record, and negative wave, *n*, of current speed in the upper record) an efferent spike, P_e, starts in the postephaptic axon.

In record I the afferent spike falls in the positive phase of the local oscillatory activity of axon B while in record II it falls in the negative phase. Note the different delays in transmission.

(*a*) *Incident impulse threshold*. With the afferent axon in good physiological state, its spike, P_a, always evoked in axon B an efferent response, P_e, (Fig. 8). The ephaptic delay, the interval between spikes P_a and P_e corrected for propagation time, is always shorter than the oscillation period of axon B. This time varies slightly and systematically, in many experiments, with the timing of the afferent spike relative to the cycle of sub-liminal activity of B: the delay is least at the maximum of the negative phase of the oscillatory potential and greatest at the maximum of the positive phase (Fig. 9; see also Fig. 8). In line with the previous discussion, since the absolute intensity of the stimulus (the afferent spike) remains unchanged, the fluctuation of

the ephaptic delay expresses the fluctuation of the excitability of the effector axon. And as the time variations follow closely the potential oscillations, this is evidence of an oscillatory variation of excitability. The existence of oscillatory variations of excitability has been also demonstrated after the make response of a fiber to a constant rheobasic current, and following the recovery from absolute refractoriness (period of the order of 5 msec.) (44); and after a brief sub-threshold shock to a citrated frog nerve (64).

Thus, in all the cases so far considered, whether the postephaptic axon is initially at rest or in oscillatory sub-liminal activity, when the afferent spike is great enough to constitute a threshold stimulus it evokes an effector response after an ephaptic delay which is determined principally by the development of the local postephaptic response. The local potential acts as the intermediary mechanism between the afferent and the postephaptic spike, just as it does between an electric stimulus and a spike.

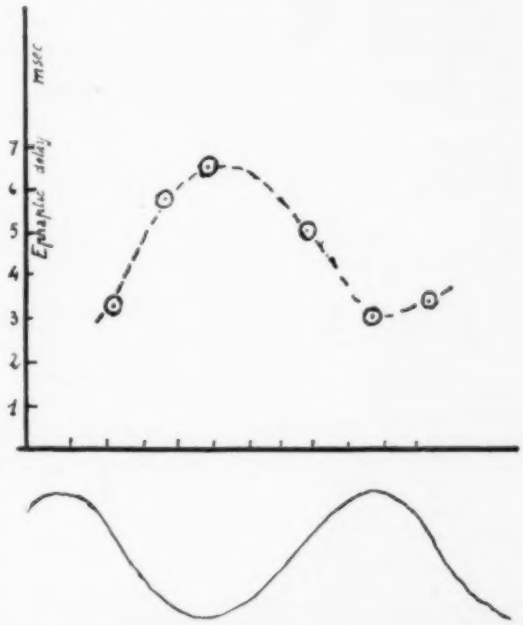

FIG. 9. Variation of the ephaptic delay with the timing of the afferent spike in the potential cycle of the postephaptic axon B, traced below.

(*b*) *Incident impulse sub-thresh old—long and variable transmission delays.* The state of the afferent axon sometimes leads to a progressive depression of its spike potentials. It is then possible to apply a graded series of spikes to a postephaptic axon which is an oscillatory activity.

When the amplitude of the afferent spike is too feeble to evoke an immediate postephaptic spike, it nevertheless brings about a progressive increment of the sub-liminal oscillations of the effector axon. The more depressed the afferent spike, the less is this increment (Fig. 10*c*, *b*, *a*). The incrementing oscillations may finally suffice to set up a postephaptic spike, after a highly variable delay (Fig. 10*c*, *b*), or they may terminate without doing so (Fig. 10*a*). Since a spike ordinarily starts at the top of a negative wave of the oscillatory potential, long ephaptic delays differ by a whole number of subliminal oscillations; *i.e.* by essentially constant time increments equal to the period of the oscillations. The delayed transmission observed by Jasper and Monnier (56) across crustacean nerves probably is of this type. The average oscillation period of a crab nerve is about 10 msec. (6), and two periods would approximate a reported delay of 22 msec.

Similar long transmission times can be seen when the afferent axon is perfectly normal and yields strictly constant spikes, providing the postephaptic axon is temporarily depressed. In Fig. 11, III, *e.g.*, the postephaptic axon has just spontaneously emitted a spike, P_{sp_1}, (seen as spread, *e*, in the record from Axon A) when an afferent spike, P_a, reaches it. Under those conditions P_a is a sub-threshold stimulus and produces only gradual incrementing of the sub-liminal activity of the postephaptic axon; this, however, after three oscillations gives rise to an efferent spike, P_e.

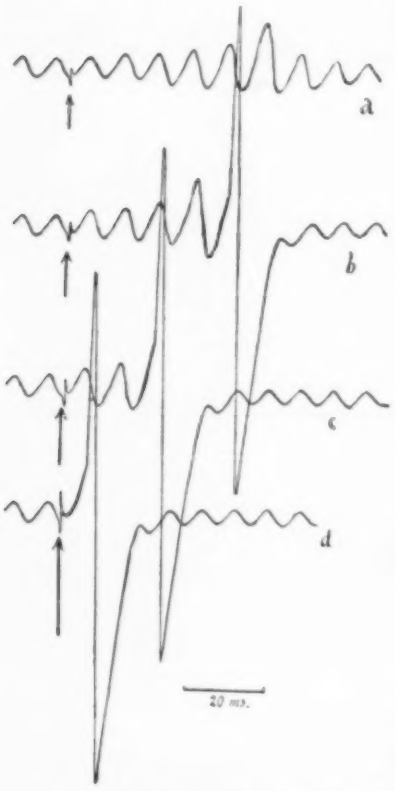

FIG. 10. Reactions of the postephaptic axon to an afferent spike of decreasing height, *d* to *a*. The arrows indicate arrival of the afferent spike.

The great latency of response, up to several hundred milliseconds, to constant currents or even condenser discharges, which is often encountered in invertebrate nerves (17) and occasionally in myelinated ones (43), is a similar phenomenon. Also, when one stimulus gives no spike, a second may sum its effect over 200 to 300 msec. (7). These and other facts show that the electric stimulus initiates slow processes, probably metabolic, which lead to augmented oscillations and final discharge of a spike.

B. The postephaptic axon is in an initial state of rhythmic liminal activity

In the case just examined the postephaptic axon was in a sub-liminal oscillatory state. Such sub-liminal activity may persist for a considerable time after repeated spike discharges, which in turn can be initiated by a single preephaptic impulse. At the beginning of such an autonomous discharge (11), a single spike may arise from the crest of each negative local wave (phase of homorhythmic activity), or (phase of heterorhythmic activity) groups of spikes are emitted, each from the crest of a negative wave but with intervening oscillations with no spikes. After discharge of a group of spikes the oscillations are much reduced and only gradually increment until another spike group can be set off.

In Fig. 11, I, axon B is autonomously rhythmic and several sub-liminal oscillations intervene between spikes. For some seconds an activity cycle was repeated with 13 to 16 oscillations, of minimal amplitude immediately after a spike and gradually increasing until the following spike is emitted. Axon A is inactive, although the escape current, e, from a spike in B (P_{sp})

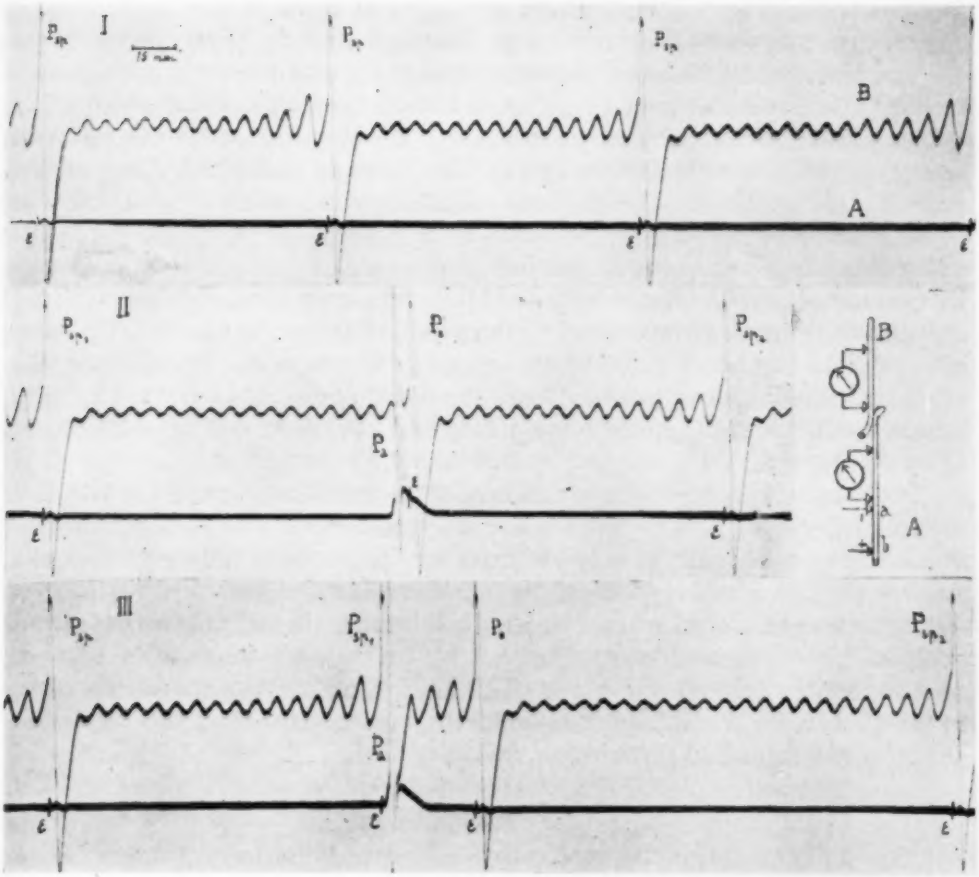

FIG. 11. Disturbances introduced by an afferent spike in the spontaneous activity of axon B (upper records):

I. Oscillatory activity of axon B, periodically becoming liminal. Only the current escapes, e, of the spontaneous postephaptic spikes, are seen in the lower record from the completely quiescent preephaptic axon, A. This activity has lasted several seconds with the interval between spontaneous spikes approximately constant at 13 to 16 sub-liminal oscillations. The events described in II and III have been superimposed on a spontaneous activity of this kind.

II. An afferent spike, P_a, evoked at *ab*, reaches the ephaptic region 16 sub-liminal oscillations after a spontaneous spike, P_{sp_1}; when axon B is due to start a second spontaneous spike. An efferent spike, P_e, starts after a delay of 3 msec., thus substituting for the spontaneous spike. The subsequent cycle of rhythmic activity of axon B is not disturbed; a new spontaneous spike, P_{sp_2} starts after 14 sub-liminal oscillations.

III. An afferent spike, P_a, evoked at *ab*, reaches the ephaptic region shortly after the emission of a spontaneous spike, P_{sp_1}. It evokes an efferent spike, P_e, after three sub-liminal oscillations of the prepotential. The rhythm of subsequent cycles is disturbed, the next spontaneous spike, P_{sp_2}, being emitted after an interval of 22 sub-liminal oscillations.

is seen in a record from it. Stimulation of A imposes an afferent spike at some point in the cycle of B and evokes an efferent spike. The transmission delay is long if the afferent spike arrives in the depressed period just after a spon-

taneous spike (Fig. 11, III), short if it arrives later (Fig. 11, II); and in either case it disturbs the cycle of spontaneous activity of B.

The interval I″ between the extra-spike, P_e, and the next spontaneous spike P_{sp_2} is not equal to the regular interval I between spontaneous spikes, but is an inverse function of the interval I′ between the previous spontaneous spike, P_{sp_1}, and the extra-spike. The extreme cases are illustrated in Fig. 11. In Fig. 11, III the incident spike P_a arrives shortly after emission of the spontaneous spike P_{sp_1}, brings about a quick increment of the height of the following subliminal oscillations, and so starts an extra response, spike P_e, prematurely in the cycle. Interval I′ includes only three sub-liminal oscillations and interval I″ includes 22 sub-liminal oscillations, about 1.5 I. When interval I′ is greater the consecutive cycle I″ is proportionally shorter. The other extreme is shown in Fig. 11, II. I′ is 16 cycles and I″ is 14; the spontaneous spike due to appear is suppressed and the next one appears after a 14 cycle interval.

Thus an early extra-spike is followed by a compensatory pause (I″–I) in the following cycle. But I′+I″ is usually less than 2 I so the spontaneous rhythm is resumed out of step with its earlier portion. A late extra-spike, however, supplants the spontaneous one soon due, presumably discharging the same accumulated excitation, and does not disturb the spontaneous rhythm. The comparable interruption by an afferent impulse of a motoneuron rhythm (Eccles and Hoff, 1932; Hoff, Hoff and Sheehan, 1935) may be recalled. Also, the ability of an incoming impulse to throw out of phase a group of synchronized elements is relevant here.

Discussion

The phenomena demonstrated on the experimental axon-axon contact can be directly transferred to the central synaptic problem, after consideration of the particular reactive characteristics to currents of the receptor element, neuron soma, and of the specific geometrical conditions of the synaptic contact. The latter are, of course, more complex than for the tangential axon-axon contact, and the asymmetry between terminal knob and cell body or dendrite may determine irreciprocal conduction by the difference in stimulating efficiency of the electric fields from one element on the other (compare case II and case III, Fig. 4). The action currents of multiple afferent knobs acting on a single receptive surface would similarly make for unidirectional conduction.

As regards the electric reactivity of the soma, certain data are available. The somata (spinal cord, superior cervical ganglion, oculomotor neurons) respond to an afferent volley by a considerable early negativity which is apparently longer than the spike (66, 54, 37, 22, 23, 40, 61, 30). In certain cases it appears to be followed by a positive phase (66, 54, 37, 40, 62). The reactions evoked by two spikes in the same afferent fibre are cumulative (24, 46). Gradation of the electric or mechanical response may be due to the addition of new units; in fact, although authors unanimously admit the

existence of a state of central excitation of essentially graduated character, they generally do not invoke a central graduated electric response. The chief cause of this abstention appears to have been the lack of demonstration of such a response of the peripheral myelinated nerve (see 5, 44, 62). However, since the functional specialization of a cell body is for accumulating, integrating and modifying the effects of stimuli, this small structure may well have different electrical properties than the long axon specialized for conduction. Actually the graduated prepotential may be the best counterpart of the soma's electric response, as suggested by records from central neurons (5, Fig. 6, B; 2). When few, or one, cortical neurones participate in an injury discharge, the spontaneous waves closely resemble the oscillations in invertebrate axons. In the visceral ganglia of *Aplysia*, large cells (200 to 300μ diameter) are easily distinguished amongst numerous ones of small dimensions (18), and these are favorable for observing the electric reactions of a single cell initially at rest to afferent impulses (34). This electric somatic response is a graduated one and shows the types seen in the axon, from an aperiodic negative wave to damped or sustained oscillations.

Thus the soma is probably the normal locus of the neuron exclusively specialized for local and graduated activity, although the axon can behave similarly at a point of stimulation. The ephapse behavior then throws light on some facts about synapses.

The brief excitation state (detonator action), produced by an afferent impulse arriving at the synapse, and evidenced by a summation period (61), is doubtless brought about by the electric field of the impulse arriving in a terminal knob. This process contributes to the summation of impulses arriving quite or almost simultaneously along different afferent fibers reaching the same soma (38, 62). It may be emphasized that this is a passive electric phenomenon, resulting from currents created by the advent of the afferent spike and by means of which it stimulates. No active response of the cell is involved and so no electric change in it—except by passive spread. The graded response of the soma follows, with its activity. What its character will be depends on whether the afferent impulse reaches the soma when this is spontaneously active (as in cortical neurons), in which case the phenomena described in part II will follow; or when it is at rest, in which case the part I effects follow—an aperiodic negative wave (as in cord and ganglion cells of vertebrates and *Aplysia*) or a train of damped oscillations (as in *Aplysia* and perhaps in deeply anaesthetized cortical cells). When the somatic depolarization is sufficient, the longitudinal potential gradient from the soma to the emerging axon initiates an efferent spike. If the liminal value is not reached "d'emblée," sub-liminal electric and excitability changes occur, as indicated by data on summation intervals. The classical optimum summation interval of 8 to 10 msec. (41, 32, 33) would be due to the first sub-liminal negative wave.

Summation at successive negative maxima is obviously difficult to detect due to the many disturbing factors, yet some evidence for it exists in a

second and more extended (due to partly out-of-phase negativity in different neurons) optimum summation interval (41, 33, 26).

In normal intact nerve the propagated activity of one axon submits each point of adjacent axons to the action of triphasic transient currents (Fig. 4, I), which cannot readily excite because of the final positivity. Moreover the interposition of myelin and of interstitial tissues between the functional surfaces efficiently insulates them from local currents. During normal propagation, therefore, the influence on neighboring fibers should be negligible. But if propagation is stopped at a cut or blocked region of the active axon, the conditions (case II of Fig. 4) favor stimulation of contiguous fibers; especially if these have been subjected to staling, alcohol, or other factors which diminish the insulating efficacy of their myelin. The synchronization and ephaptic phenomena do occur in nerve under just these conditions.

If the local sub-liminal activity itself constitutes a significant stimulus to neighboring axons, as experiments to be reported elsewhere show it does, a peculiar quasi-sinusoidal form of physiologically functional stimulus becomes important. Such a stimulus is liable to variation of frequency and amplitude under the influence of many factors (12) and so introduces many new possibilities of electric action at junctions, beside the simple brief, monophasic, all-or-nothing spike potential. Such considerations apply also to central cell bodies, especially since these have little lipid insulation at their surfaces (25, 35); and the existence of ephaptic interactions between cell bodies seems highly probable. The same factors which favor axon synchronization also favor central synchronization. Synchronization by synaptic paths cannot be formally excluded, but strong evidence indicates the efficacy of ephaptic interactions and their predominance in abnormal cases. Direct experimental evidence for this is seen in the synchronization of retinal neurons (5a) and of those of the olfactory bulb of the isolated frog brain following synaptic paralysis by nicotine (59; *Ed.:* see also 50, 51).

Study of the experimental axon-axon contact should thus throw light on liminal and sub-liminal synaptic phenomena and on the more general ephaptic interactions determined by current flow between near-by elements, which contribute to transmission and coordination.

Summary

Several conditions of contact (ephapse) between two isolated *Sepia* axons permit study of the electric reactions evoked in one (initially active or at rest) by the electric activity of the other.

The current spread from the active axon serves as a polyphasic electrical stimulus to the contiguous axon. The general appearance, height and duration of this stimulus depend, on the one hand, on the shape, height and propagation speed of the action potential of the active fiber; and, on the other, on the geometrical conditions of contact and on the excitability characteristics of the receptive axon.

When the contact conditions are such that the currents penetrating the

resting axon have a large final positive phase, they produce no visible effect on it. When this final positive phase is sufficiently depressed, the effects of the preceding negative phase predominate and evoke characteristic subliminal or liminal active electrical reactions of the resting axon. This case is realized for the portion of the resting axon in contact with a termination of the active axon and to a spike ending there. The phenomena in such an experimental arrangement have been observed and analyzed in detail. The active axon which conducts an impulse towards the contact region has been called the afferent or preephaptic axon; the axon acted upon by it, the efferent or postephaptic axon.

When contact conditions are such that the action currents of the afferent impulse constitute only a subthreshold stimulus to the efferent axon, this responds with a local potential of variable height and either aperiodic or with damped oscillations. When the preephaptic action currents reach a threshold value a spike is discharged along the postephaptic axon, 2 to 5 msec. after the afferent spike reaches the contact zone. This interval is occupied by the rise of the local active prepotential to a threshold value. Depending on the state of the effector axon, an after-discharge may or may not follow this first spike.

Additional phenomena appear when the efferent axon displays an initial oscillatory activity of its own. In this case, a subthreshold afferent spike brings about incrementing of the spontaneous oscillations of the efferent axon; and often, after a few such increasing oscillations, the discharge of a spike. As the stimulating value of the afferent impulse increases to threshold, the delay at the ephapse shortens and finally becomes less in the cases considered in the preceding paragraph. If the autonomous activity of the efferent axon includes a periodic discharge of spikes, the introduction of an extra-response, by means of an afferent spike, causes a break in this rhythm. An early extra-response is followed by a prolonged cycle, as if there were a compensatory pause.

The ephaptic phenomena have been discussed in relation to synaptic phenomena and to other interaction phenomena between central cells, particularly those involved in synchronization.

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LOCALIZATION OF ENZYMES IN NERVES
I. SUCCINIC DEHYDROGENASE AND VITAMIN B_1

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INTRODUCTION

RECENT investigations have shown that the enzyme choline esterase is found in high concentrations in the sheath of the giant axon of the squid, only a negligible amount being present in the bulk of the axoplasm (2, 14). This finding is of particular interest in view of the parallelism found between electrical activity and concentrations of the enzyme in electric organs, and suggests that there is a correlation between acetylcholine metabolism and electrical activity in general (15).

In view of the high concentration of choline esterase at the neuronal surface it becomes important to determine the distribution of other enzyme systems known to be of consequence in the general metabolism of the cell as well as in the specific processes probably involved in excitation and transmission. The present paper deals with the distribution in squid nerves of succinic dehydrogenase and oxidase systems and with the vitamin B_1 in its phosphorylated form, diphosphothiamin.

METHODS

a. *Succinic dehydrogenase.* The method of Quastel and Wheatley (1938) has been used. The test depends on the reduction of ferricyanide by the hydrogen of succinic acid. For one reduced molecule of ferricyanide one molecule of acid is formed, and in bicarbonate solution this gives rise to one molecule of CO_2, which is estimated manometrically. According to the equations for the reactions involved:

$$H + Fe(CN)_6^{\cdots} \rightarrow H^+ + Fe(CN)_3^{\cdots}$$
$$H^+ + HCO' \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

each molecule of succinic acid will liberate two molecules CO_2.

The amounts of tissue available were small and, at room temperature, lower rate of reaction could be expected than in the work of Quastel and Wheatley. In order to increase the sensitivity of the method, small conical vessels with side bulb were used with a total volume of about 7 cc. The KCO_2 of these vessels at room temperature was 0.6–0.7, if about 1 cc. fluid was present. In the main compartment of the vessel there was placed 1 cc. of Ringer-$NaHCO_3$ (0.025 M), and in the side bulb 0.1 cc. of the ferricyanide solution (5 cc. of a 10 per cent sodium ferricyanide solution with 1 cc. 0.16 M $NaHCO_3$). The gas mixture used was 95 per cent N_2 + 5 per cent CO_2. All experiments were carried out at 22–23°C.

Sheath and axoplasm samples of giant fibers of the squid were always prepared from two pairs of fibers, one used as control without succinate the other with succinate. The separation of sheath and axoplasm was carried out as previously described (18). Samples were weighed on a microbalance and then finally minced in the manometer vessels. Only the difference of the CO_2 output between experimental and control value was considered as a measure of the activity of succinic dehydrogenase.

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b. *Succinic oxidizing system.* Oxygen uptake was determined with the same vessels as used for succinic dehydrogenase and the material was prepared in the same way. As buffer, 1.5 cc. of a 0.1 M phosphate solution were used with a *p*H of 7.3. The succinate concentration was always 0.15 M. 1.5 mg. of cytochrome dissolved in 0.1 cc. were added to each vessel in order that an insufficient concentration of cytochrome should not be the limiting factor. The cytochrome was prepared as described by Keilin and Hartree (6). The determinations were carried out in air. The difference of the O_2 uptake with and without succinate was considered as a measure of the activity of succinic oxidizing system.

c. *Cocarboxylase (diphosphothiamin).* The method of Lohmann and Schuster (9) was used. This method is based on the principle that the carboxylase in washed yeast extract will decarboxylate pyruvic acid only in presence of diphosphothiamin. If to a washed yeast preparation which has practically no decarboxylating action, a tissue extract is added and the CO_2 output compared with the CO_2 output in the presence of known concentrations of diphosphothiamin, the amount of this substance in the extract can be calculated by interpolation.

The method has been used with slight modifications of the original. 1 g. well dried and ground yeast was weighed into a centrifuge tube and about 50 cc. H_2O were added. The tube was shaken vigorously at intervals for 20 min., after which the suspension was poured into another centrifuge tube and centrifuged for 5 min. and the supernatant fluid discarded. The yeast was then washed twice with about 50 cc. of 0.1 M dibasic phosphate at 30°C., and then once with distilled water. Each of the three washings was carried out within 4 min., centrifuging included. 8 cc. of phosphate buffer of a *p*H of 6.2 were then added and 0.8 cc. of this suspension put into each vessel together with 1 cc. of extract or known diphosphothiamin solution.

In the side bulb were placed 0.2 cc. of a solution, containing 0.2 mg. Mg, 10μg. thiamin and 5.0 mg. sodium pyruvate. This latter was freshly prepared for each experiment: about 50 mg. were weighed and a corresponding amount of the stock solution of Mg and thiamin were added. After the temperature equilibrium was reached the solution of the side bulb was tipped into the main vessel. The reading after the first 5 min. was discarded.

Results

A. *Succinic dehydrogenase*

The ultimate source of energy used in living cells is oxidation and most cellular functions consume energy. Conduction of nerve impulses requires energy: it is connected with heat production and extra oxygen uptake (3, 4, 5). But two restrictions have to be made: (i) Oxidation is a slow process. It is known, from investigations on muscle, that oxidation is not connected directly with contraction but occurs during recovery (12). It is difficult to believe that the extra oxygen uptake in nerves is directly connected with conduction, an event far more rapid than muscular contraction. (ii) The extra oxygen uptake during nerve activity is small compared with the oxygen uptake of the resting nerve, in contrast with the important extra oxygen uptake following muscular activity. Whatever the role of oxidation in conduction may be over all oxygen consumption is certainly connected with many other activities of the nerve cell. It therefore can be expected that the localization of respiratory enzyme systems differs essentially from that of choline esterase.

It is widely believed that succinic dehydrogenase is an essential intermediate in respiration. The work of Szent-Györgyi (19) and his associates suggests that the succinic-fumaric acid system is directly linked to the cytochrome-cytochrome oxidase system. The activity of succinic dehydrogenase has been determined in the head ganglion of squids, where cell bodies

Table 1. Succinic dehydrogenase in the head ganglion at different concentrations of succinate, varying from 0.075 to 0.30 M. All figures are calculated for 100 mg. fresh tissue.

	Control 30.5 mg.	0.075 M 25.0 mg.		0.15 M 26.0 mg.		0.30 M 26.0 mg.	
min.	cmm. CO_2	cmm. CO_2 (corr.)	μg. succinic acid	cmm. CO_2 (corr.)	μg. succinic acid	cmm. CO_2 (corr.)	μg. succinic acid
70	60.0	206	*542*	228	*600*	199	*524*
185	95.0	431	*1135*	472	*1240*	415	*1090*

and synapses are located, in whole giant axon and in sheath and axoplasm separately. The enzyme activity was also determined in the whole trunk containing, besides the giant axon, many fibers of small diameter and therefore relatively larger surface.

Table 1 gives the values obtained with the head ganglion. The control values indicate the CO_2 liberation per 100 mg. of fresh tissue without

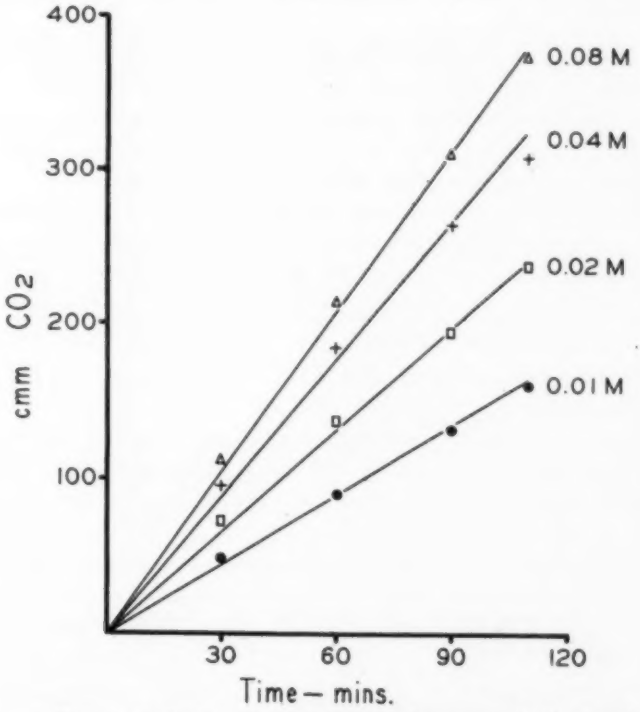

FIG. 1. Activity of succinic dehydrogenase in the head ganglion of the squid at different concentrations of succinate. Abscissae: cmm. CO_2 liberated by 100 mg. fresh tissue (corrected). Ordinates: time of observation in min.

succinate. From the experiments with succinate are given the figures of CO_2 production calculated for 100 mg. of fresh tissue after subtraction of the

Table 2. Succinic dehydrogenase in the whole giant axon of squids.

Exp. Nr		Control 22.0 mg.	Experiment 23.0 mg.		
		cmm. CO_2	cmm. CO_2	μg. succinic acid	
	min.	per 100 mg.	per 100 mg. (corr.)	absol.	per 100 mg.
1	80	7.4	21.3	*12.9*	56.0
	160	10.3	44.9	*27.1*	118.0
	255	13.3	65.5	*39.6*	172.0
	320	14.8	78.2	*47.3*	206.0
		Control 30.0 mg.		Experiment 28.0 mg.	
2	80	6.6	19.1	*14.1*	50.2
	160	9.8	40.9	*30.1*	107.5
	230	9.8	54.8	*40.5*	144.5

control and the corresponding amount in μg. succinic acid dehydrogenated. At a concentration of 0.01 M of succinate used by Quastel and Wheatley the activity rate in these experiments is far from being optimal (Fig. 1). The values in Table 1 indicate that 0.15 M is about optimal. This concentration was used in experiments with the giant fiber.

The activity in the giant fiber is considerably smaller, less than 10 per cent of that in the head ganglion. In the whole giant axon about 50 μg. of succinic acid were metabolized in 80 min. at 0.15 M concentration per 100 mg. fresh tissue as compared with 670μg. in the head ganglion during the same time, at the same substrate concentration and the same temperature (Table 2). In the whole trunk the amount was even smaller: only 37 μg. per 100 mg. fresh tissue (Table 3).

If the activity of succinic dehydrogenase is determined in sheath and axoplasm separately, the most important fact is that in absolute amounts about 90 per cent of the enzyme is located in the axoplasm (Fig. 2). The

Table 3. Succinic dehydrogenase in the nerve trunk containing the giant axon.

	Control 94.0 mg.	Experiment 83.0 mg.		
	cmm. CO_2	cmm. CO_2	μg. succinic acid	
	per 100 mg.	per 100 mg. (corr.)	absol.	per 100 mg.
80 min.	7.0	14.0	*31*	37
160 min.	10.7	25.0	*55*	66
230 min.	15.2	30.3	*65*	80

values obtained with sheath are in fact so small that they are within the limits of sensitivity of the method. In Table 4 the observed CO_2 values are given and not those calculated for 100 mg. of tissue as in the previous tables, because in this case the absolute amounts are essential. The figures show that

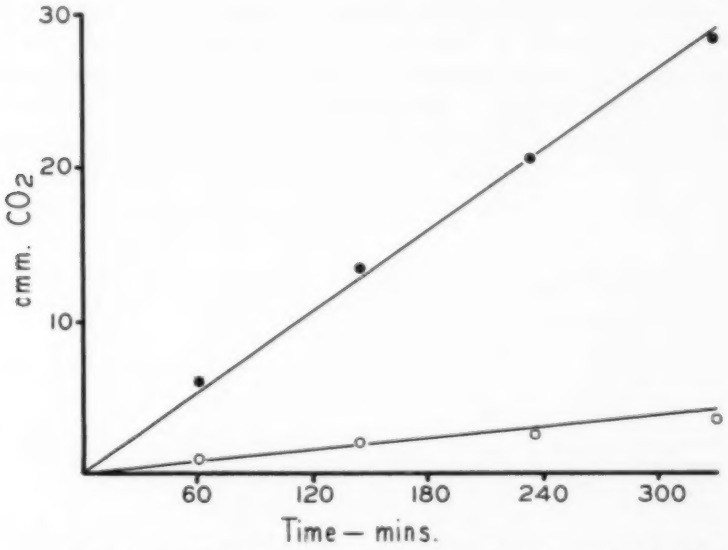

FIG. 2. Succinic dehydrogenase in sheath and axoplasm of the giant fiber of the squid. Abscissae: cmm. CO_2 liberated. Absolute amounts (corrected). Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

Table 4. Succinic dehydrogenase in sheath and axoplasm of the giant fiber of squids.

		Sheath				Axoplasm			
		Control 5.6 mg.	Experiment 5.2 mg.			Control 17.6 mg.	Experiment 20.9 mg.		
	min.	cmm. CO_2 observ.	cmm. CO_2 observ.	μg. succinic acid		cmm. CO_2 observ.	cmm. CO_2 observ.	μg. succinic acid	
				absol.	per 100 mg.			absol.	per 100 mg.
I	80	0.65	1.50	*2.3*	44	3.8	9.9	*14.1*	67.5
	145	1.63	2.41	*2.3*	44	5.5	15.8	*24.3*	116
	235	1.95	2.71	*2.4*	46	7.6	23.6	*38.6*	185
		Control 6.7 mg.	Experiment 5.5 mg.			Control 27.5 mg.	Experiment 24.9 mg.		
II	60	0.65	1.50	*2.6*	47	2.7	8.5	*17.9*	72
	145	1.63	3.31	*5.2*	95	3.8	17.0	*35.5*	142.5
	235	1.95	4.21	*6.9*	126	4.5	24.5	*53.8*	216
	330	1.95	5.41	*9.3*	169	5.2	32.8	*73.7*	296

the enzyme activity in the sheath is hardly measurable. If we accept them as an approximate indication of the enzyme activity in the sheath the concentration (amount metabolized per 100 mg. wet weight) is about half of that in the axoplasm. A considerable part of the sheath is connective tissue, which has no enzymatic activity. Some axoplasm may remain attached to the sheath although most of it has been squeezed out. It is still possible that the actual concentration of the enzyme is somewhat higher in a very thin layer at or near the nerve surface. These experiments do not offer any evidence for such a possibility. The same considerations can be applied to the low values obtained with the whole trunk which contains on the one hand small fibers with a relatively large surface and on the other hand more connective tissue than the giant axon. It is, however, certain that the distribu-

Table 5. Succinic oxidizing system in the head ganglion of squids. All figures are given for 100 mg. of fresh tissue. C = Control, E = Experiment, S.A. = succinic acid.

Exp. No.:	I			II			III			IV			V		
	C 36.5 mg.	E 43.5 mg.		C 42.5 mg.	E 42.5 mg.		C 40.0 mg.	E 38.5 mg.		C 34.5 mg.	E 38.0 mg.		C 33.5 mg.	E 35.0 mg.	
min.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.
60	14.0	116	*0.61*	18.5	94.5	*0.50*	16.2	115	*0.60*	17.8	105	*0.55*	11.6	110	*0.58*
120	19.0	211	*1.11*	26.0	183	*0.96*	24.3	211	*1.11*	26.8	203	*1.07*	17.9	225	*1.18*
180	—	—	—	—	—	—	30.0	297	*1.56*	34.6	288	*1.52*	22.5	322	*1.69*
240	26.6	350	*1.84*	38.4	326	*1.72*									

tion of succinic dehydrogenase is in striking contrast to that of choline esterase.

B. *Succinic oxidizing system*

The final oxidation of succinate requires, besides succinic dehydrogenase and other intermediate links, the cytochrome-cytochrome oxidase system. Succinic dehydrogenase is the limiting factor of the rate of the reaction, but as in most cells it is present in rather large amounts, oxidation of succinic acid may give an approximate indication for the distribution of cytochrome oxidase. It shows in any case whether succinic acid can be oxidized at the same rate as it is dehydrogenated.

In five experiments oxidation of succinic acid has been determined in the head ganglion (Table 5). Cytochrome c was added in excess thus ensuring that an insufficient concentration of this substance would not be a limiting factor. About 500–600 μg. succinic acid were oxidized in 60 min. by 100 mg. fresh tissue. This amount corresponds to the amount dehydrogenated by succinic dehydrogenase in anaerobiosis. It is calculated on the assumption that one molecule oxygen oxidizes one molecule succinic acid. This means that the reaction stops with the formation of hydrogen peroxide, no catalase

being present to split the H_2O_2. No experiments have been made to test whether catalase is present or not. But as it is hardly possible that two molecules of succinic acid are oxidized for 1 molecule dehydrogenated, the calculation based on the assumption of H_2O_2 formation appears justified.

The activity of the succinic oxidizing system in sheath and axoplasm of the giant fiber is the same as that found for succinic dehydrogenase. The calculation is again based, as for the head ganglion, on the assumption of H_2O_2 formation. Only one experiment has been made due to shortage of material at the advanced season when the experiments were made. But it appears significant as all experiments with the head ganglion preparations show that the amounts of succinate metabolized are the same in aerobic and anaerobic conditions and there is no reason for assuming that the ratio of the two enzymes in the fiber is different. Figure 3 demonstrates that the oxidation of succinic acid in the giant axon parallels the rate of its dehydrogenation shown in Fig. 2.

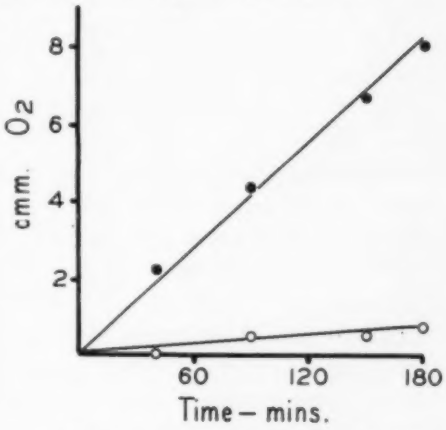

FIG. 3. Succinic oxidizing system in sheath and axoplasm of the giant fiber of the squid. Abscissae: absolute amounts of O_2 taken up (corrected). Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

C. *Vitamin B_1 (Diphosphothiamin)*

Oxidation of pyruvic acid goes through formation of acetic acid (1, 8, 13, 16). Acetylation of various substances occurs easily subsequent to pyruvic acid oxidation. It is therefore probable that the acetic acid of acetylcholine is derived from pyruvic acid. Evidence for this assumption was brought out in experiments of Quastel and his colleagues who found that formation of acetylcholine in brain slices occurs only in oxygen in presence

Table 6. Succinic oxidizing system in sheath and axoplasm of the giant fiber of squids.

	Sheath				Axoplasm			
	Control 4.1 mg.	Experiment 3.7 mg.			Control 22.1 mg.	Experiment 22.4 mg.		
min.	O_2 cmm. observ.	O_2 cmm. observ.	μg. succinic acid		O_2 cmm. observ.	O_2 cmm. observ.	μg. succinic acid	
			absol.	per 100 mg.			absol.	per 100 mg.
40	1.05	0.97	*0.12*	3.16	1.13	3.43	*12.0*	54
90	1.05	1.45	*2.64*	71.8	1.40	5.81	*23.1*	103
150	1.84	2.17	*2.69*	72.8	1.97	8.71	*35.4*	158
180	1.84	2.41	*3.94*	107.0	2.25	10.30	*42.4*	189

Table 7. Cocarboxylase in head ganglion and giant axon of squids.

Exp. No.	tissue	mg. fresh tissue	μg. cocarboxylase	
			absol.	per 100 mg.
1	head ganglion	159.0	2.10	*1.32*
2	head ganglion	176.0	2.15	*1.22*
3	2 head ganglia	328.0	4.14	*1.26*
4	whole giant-axon	52.7	0.055	*0.105*

of either glucose or pyruvate. Since it is well known from the work of Peters and his associates that the oxidation of pyruvic acid requires vitamin B_1, Mann and Quastel (11) investigated whether the formation of acetycholine in brain slices, kept in pyruvate-Locke-bicarbonate media, is accelerated in presence of vitamin B_1. In brains of normal pigeons vitamin B_1 had no effect, but in vitamin deficient pigeons addition of vitamin B_1 increased definitely the rate of acetylcholine formation, although only in presence of a high potassium concentration (0.03 M).

If acetylcholine formation requires vitamin B_1 it appeared possible that, in view of the high acetylcholine metabolism at or near the surface of nerve fibers, vitamin B_1 was concentrated there as well. However, it would not be expected that vitamin B_1 is localized near the surface as exclusively as choline esterase because pyruvic acid oxidation is an important intermediate step for many reactions other than acetylcholine formation.

The active form of vitamin B_1 in cells is the diphosphothiamin (cocarboxylase) and most of the vitamin B_1 in tissues seems to occur in this form (8). The cocarboxylase has been determined in the head ganglion and in sheath and axoplasm of the giant fiber in order to compare its distribution with that of choline esterase. Extracts were prepared of sheath and axoplasm by grinding in quartz tubes with quartz powder and water. The tubes were

Table 8. Cocarboxylase in sheath and axoplasm of the giant fiber of squids.

	Control	Sheath 14.0 mg.			Axoplasm 87.0 mg.			Standard 0.05 μg. coc.	
	CO_2 c.mm.	CO_2 c.mm.		μg. coc.	CO_2 c.mm.		μg. coc.	CO_2 c.mm.	
min.	observ.	observ.	per 100 mg. (corr.)	absol.	observ.	per 100 mg. (corr.)	absol.	observ.	corr.
10	1.00	2.36	9.7	*0.0356*	4.09	3.6	*0.0807*	2.91	1.91
15	1.66	3.84	15.6	*0.0342*	6.81	5.9	*0.0807*	4.85	3.19
20	2.66	5.32	19.0	*0.0303*	9.52	7.9	*0.0781*	7.05	4.39
25	3.65	6.80	22.5	*0.0310*	11.6	9.2	*0.0784*	8.73	5.08
30	3.98	8.00	28.7	*0.0299*	14.0	11.5	*0.0746*	10.70	6.72
average value of 5 readings:				*0.0322*			*0.0785*		
per 100 mg:				*0.230*			*0.090*		

then immersed in boiling water for 3 min. following which they were stored at low temperature until used.

1.2–1.3 μg. cocarboxylase per 100 mg. fresh tissue have been found in the head ganglion (Table 7). This is a value several times as high as that of Ochoa and Peters for pigeon's and rat's brains (2.5–4.0 μg. per g. fresh tissue). In the the whole giant axon the value found was rather low, less than 10 per cent of that in the head ganglion: 0.105 μg. per 100 mg. fresh tissue.

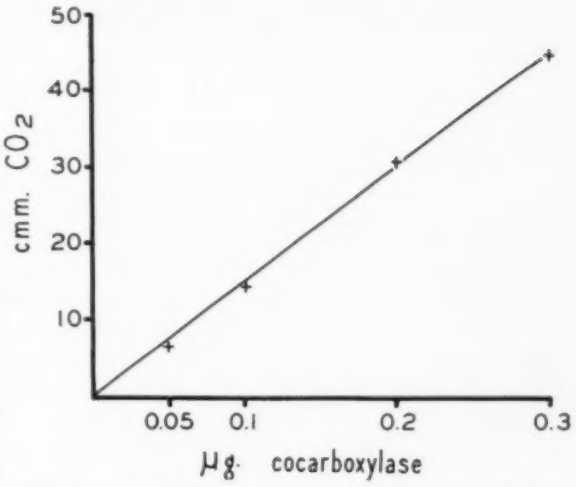

FIG. 4. CO_2 developed in 30 min. at 4 different concentrations of cocarboxylase.

In view of the low concentration of cocarboxylase in the fiber and the small amounts of tissue available only small quantities of cocarboxylase could be expected in the extracts prepared. The method had therefore to be adapted to such small quantities. This did not represent any particular difficulty (see details under methods). Table 8 gives one complete experiment. Rates calculated from readings at different times show a satisfactory agreement. If four different concentrations of the standard solution (0.05, 0.1, 0.2, and 0.3 μg.) are plotted at a given time against the CO_2 liberated, a straight line is obtained (Fig. 4). In order to test the sensitivity of the method one experiment has been carried out with amounts of 0.05, 0.07 and 0.1 μg. cocarboxylase. Figure 5 shows that even with such low quantities the difference of the various concentrations is sufficiently marked and that if the amounts of cocarboxylase are plotted against the amount of CO_2 liberated at a given time a straight line is obtained.

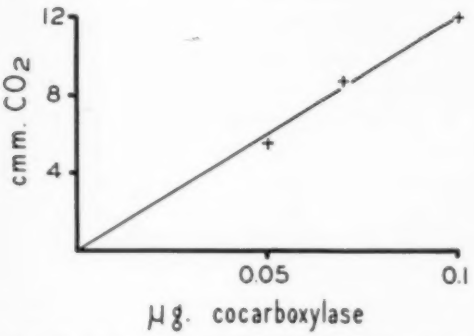

FIG. 5. CO_2 developed in 30 min. with small amounts of cocarboxylase.

The figures show that the concentration of cocarboxylase is higher in the sheath than in the axoplasm. The *absolute* amount per nerve is somewhat higher in the axoplasm because of the greater mass of material. The difference of concentration between sheath and axoplasm is shown in Fig. 6. In Table 9 the results of 4 experiments are given. The best time of observation is about 25 min. However, the first experiment was continued only 15 min.

after substrate and enzyme were mixed and in the second experiment no reading was made at 25 min. All four experiments show that the concentration of cocarboxylase in the sheath is about 2–3 times as high as that in the axoplasm. In absolute amounts the figures of experiment no. 3 are lower than those of the other experiments. This is probably due to the fact that these extracts were kept for nearly a week in order to collect a maximal amount of material. It is probable that during such a long time a certain dephosphorylation occurred and that therefore the extracts yielded low values.

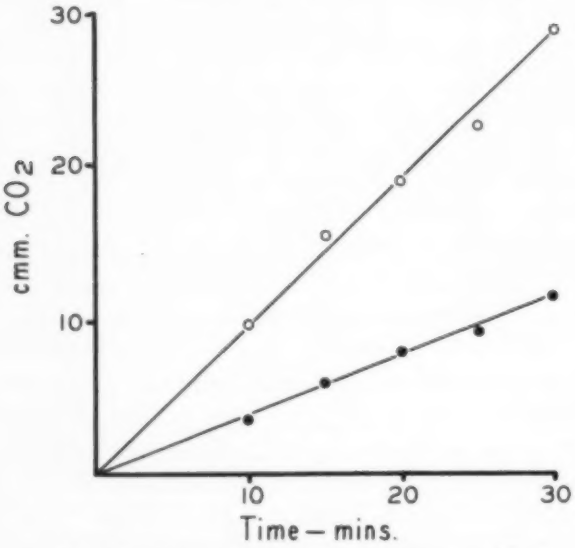

FIG. 6. Concentration of diphosphothiamin (cocarboxylase) in sheath and axoplasm of the giant fiber of the squid. Abscissase: cmm. CO_2 liberated by extracts calculated for 100 mg. of fresh tissue. Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

If the *concentration* in the sheath is 2–3 times higher than that in the axoplasm the real difference must be still greater; at least 50 per cent of the sheath used is connective tissue, probably more, and a part of the axoplasm may adhere to the sheath thereby decreasing the apparent concentration. A cautious estimate would be that

Table 9. Cocarboxylase in sheath and axoplasm of the giant fiber of squids.

Exper. No.	part of fiber	mg. fresh tissue	min. of observ.	μg. cocarbox.	
				absol.	per 100 mg.
1.	Sheath	15.2	15	0.0498	*0.327*
	Axoplasm	87.0		0.0476	*0.055*
2.	Sheath	13.1	30	0.0354	*0.270*
	Axoplasm	72.5		0.0745	*0.103*
3.	Sheath	22.5	25	0.0313	*0.139*
	Axoplasm	110.0		0.0768	*0.070*
4.	Sheath	14.0	25	0.0310	*0.221*
	Axoplasm	87.0		0.0784	*0.090*

the concentration of cocarboxylase at or near the surface is at least 5–10 times as high as in the axoplasm.

Discussion

The localization of choline esterase at or near the neuronal surface is an essential support for the conception that acetylcholine metabolism is intrinsically connected with the electrical changes occurring during nerve activity. If the localization of this enzyme is to be accepted as significant, the distribution of other enzymes less closely connected with activity should differ from that of choline esterase. The distribution of the enzymes studied so far and described in this paper supports this assumption: the greater part of succinic dehydrogenase and oxidase, widely considered as important links in the respiratory enzyme system, is located in the axoplasm. Although it is not certain whether the whole respiration passes through the succinic-fumaric acid system as originally suggested by Szent-Györgyi, it may be taken as an approximate indication for the distribution of respiratory enzymes. This agrees well with the assumption that oxidation is most probably not as closely and specifically connected with nerve conduction and transmission as acetylcholine metabolism and that oxidation, whatever its significance for conduction may be, is important for most cell activities. It is, however, desirable to test other important links, especially cytochrome oxidase, before drawing a final conclusion about the distribution of the respiratory enzymes. Lack of material, with the method used, made it impossible to study other respiratory enzymes during this season. The studies will be continued with a more adequate micro-respiration technique. In any case the contrast to the distribution of choline esterase is very marked.

The distribution of vitamin B_1 as diphosphothiamin, its active form, differs both from that of choline esterase and from that of succinic dehydrogenase. Its concentration is definitely higher in the sheath although the absolute amounts are somewhat higher in the axoplasm which contributes the greatest bulk of the whole nerve. It is suggested that this concentration is connected with the fact that oxidation of pyruvic acid is necessary for acetylation of choline, a process requiring vitamin B_1. This assumption may explain the sensitivity of the nervous system to vitamin B_1 deficiency: For if acetylcholine is essential for conduction and transmission of nerve impulses a decrease in the rate of its formation may lead to the polyneuritis observed in this deficiency.

Whatever the interpretation of this distribution may be, the fact itself, that the localization of the three enzymes studied differs so greatly, appears significant and emphasizes the specificity of the localization of choline esterase.

Summary

Studies have been initiated to determine the distribution in the nerve cell of different enzymes possibly important for nerve activity and to compare it with the distribution of choline esterase. The enzyme activities have been determined in sheath and axoplasm of the giant fiber of squids and in the

head ganglion. So far succinic dehydrogenase, the succinic oxidizing system and vitamin B_1 as diphosphothiamin have been determined.

(1) About 90 per cent of succinic dehydrogenase of the giant axon are located in the axoplasm. The experiments bring no evidence for the assumption that the enzyme is concentrated at or near the surface. The distribution is in striking contrast to that of choline esterase. In the head ganglion the concentration of succinic dehydrogenase is about 10 times as high as that in the fiber. The oxidation of succinic acid occurs at the same rate as its dehydrogenation.

(2) Diphosphothiamin is concentrated several times more in the sheath than in the axoplasm. This fact supports the assumption that vitamin B_1 is required for the formation of acetic acid for the acetylcholine from pyruvic acid. The distribution differs from that of choline esterase as well as from that of succinic dehydrogenase.

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CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF THE CEREBRAL CORTEX IN MONKEYS AND CATS*

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INTRODUCTION

ELECTRICAL stimulation in the region of the pons results in action potentials in cat's cerebellum, in lobulus ansiformis, paraflocculus, lobulus paramedianus, folium and tuber vermis, culmen, lobulus simplex and pyramis (Dow, 1939). Curtis (1940) stimulated the cerebral cortex in cats and obtained action potentials in the cerebellar cortex in the same lobules, except that none was found in paraflocculus nor the lobules of vermis posterior to lobulus simplex. The corticopontocerebellar system is best studied in primates, for in these animals this afferent cerebellar system has its greatest development. The present report deals with the responses in various lobes of the cerebellum following electrical stimulation of the major subdivisions of the cerebral cortex in monkeys and cats.

MATERIAL AND METHODS

Observations were made on 8 monkeys and 11 cats. Most of the animals were anesthetized with nembutal (1 cc. 6.5 per cent solution per 2.27 kg.) given intra-peritoneally. In a few ether anesthesia was used throughout the experiment. In the experiments in which the anterior lobe of the cerebellum was explored with the lead electrode the occipital lobe of one cerebral hemisphere was ablated and the tentorium cerebelli laid back to expose the entire culmen on that side. Stimulation was produced by a single condensor discharge of relatively short duration (0.3 msec.) delivered through an audio-transformer to reduce the shock artifact. The stimulating electrode consisted of a glass bead in which two wires were buried. The glass was then ground until the ends of the wires were flush with the flat surface of the electrode. The exposed surfaces of the wires were about 1.5 mm. apart. This was lightly applied to the surface of the brain by means of a mechanical manipulator. The strength of the shock used to explore the cortex with these electrodes was 140 V. peak voltage. This was approximately 20 times the threshold in a zone of lowest threshold. This strong shock, when delivered through the glass bead electrode, never produced visible spread to nearby muscles, and the response could be abolished by the local application of cocaine solution. When silver wire electrodes 2 to 3 mm. apart were applied to the cortex, evidences of spread of current to adjacent tissues were observed when the voltage was above 50 V. The essentially local effect of the strong shock delivered through the glassed electrodes was further shown by the fact that a movement of only 2 or 3 mm. would frequently radically change the cerebellar response and in certain areas cause it to disappear completely.

Lead electrodes consisted of a pointed wick of cotton wool whose contact surface was rarely greater than 2 mm. in diameter and usually about 1 mm. An indifferent electrode was placed on the skull or on the skin of the neck. In certain experiments two leads were taken from the same cerebellar lobule with two wicks of cotton or with two silver wires a few mm. apart. This was in order to be doubly sure that the potentials were the result of nervous activity at the lead site. In some of the experiments a double channel cathode ray

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oscillograph was used so that the separate responses at two points could be observed from an identical stimulus.

Results

Cat. Cerebellar action potentials were observed following stimulation of the opposite cerebral hemisphere in every experiment. The lobes of the cerebellum in which there were responses were culmen, lobulus simplex, lobulus ansiformis, folium and tuber vermis, pyramis, lobulus paramedianus and paraflocculus, This represents all the folia which may be exposed on the surface of the cerebellum of the cat except the uvula. Action potentials were never observed from leads on the uvula in the cat. Figure 1C shows the potentials obtained from some of these lobes. These records were taken with both leads on the same lobule of the cerebellum only a few millimeters apart. This accounts for the complex wave form in some of the leads. Although the crest of the major deflection occurred about 25 msec. after the shock artifact, it is obvious that there is local activity appearing much earlier, in fact as early as 3

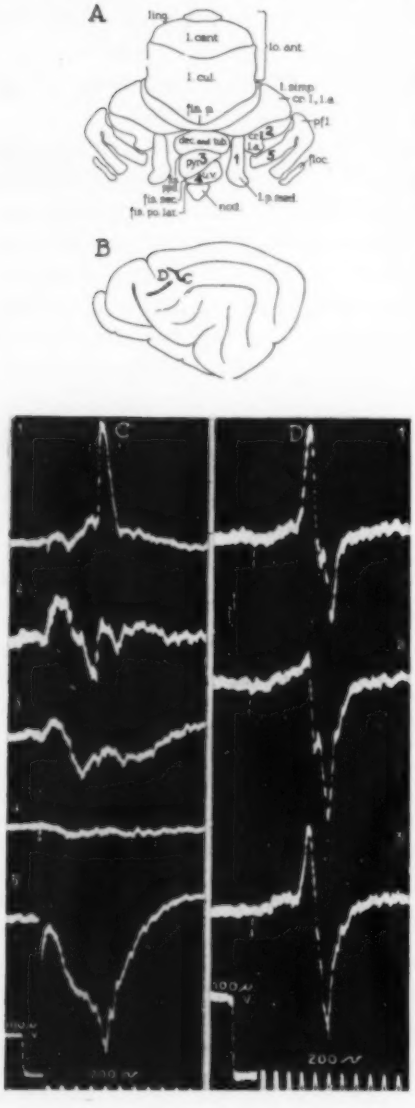


Fig. 1. A. Schema of the cat's cerebellum. Numbers 1–5 refer to corresponding numbers in C and indicate the lead points chosen for the records. Stimulus identical in strength and location. B. Diagram of the left cerebral hemisphere in the cat. C indicates stimulus point for records C 1–5. D indicates the point at which needle electrodes were thrust 4 mm. into subcortical white matter. At this depth, stimulation with low voltage A.C. current gave an isolated movement of thigh muscles. Single shock stimulation here produced action potentials shown in D. C. Bipolar surface lead on (1) paramedianus; (2) Crus II lobulus ansiformis; (3) pyramis; (4) uvula; (5) paraflocculus. D. Three responses on lobulus paramedianus from identical stimuli applied to the same subcortical point at about 4 sec. intervals. Note differences in form of each response. Bipolar recording.

msec. It was regularly observed that responses resulting from stimulation of just above threshold strength consisted only of the wave at the 25 msec. latency. As the strength of the shock was increased to several times threshold, the earlier response would then appear. Although a much later response, occurring after a latency of 200 msec. (Curtis, 1940), was frequently watched

for on slower sweep speeds, it appeared only rarely, and then was so inconsistent in appearance as to make its interpretation difficult. No statement can be made from this study concerning the presence or absence of potentials from the folia of the base of the vermis and flocculus, but following stimulation of the pons in the cat there were none in these lobules (Dow, 1939).

The sigmoid, coronal, lateral, suprasylvian, ectosylvian and sylvian gyri of the cerebral cortex were stimulated. Responses appeared in widely scattered parts of the cerebellar cortex from stimulation of a single point on the cerebral cortex. The most marked and most consistent responses and those at lowest threshold resulted from stimulation in the sigmoid, coronal, anterior ectosylvian and anterior part of the lateral, suprasylvian and middle ectosylvian gyri. A single response found only in the pyramis was obtained from the posterior ectosylvian gyrus and a point in the posterior suprasylvian gyrus. In another isolated experiment there was a response in the lobulus paramedianus from a stimulus in the posterior part of the middle ectosylvian gyrus. There were no responses on any part of the cerebellum in any experiment from stimulation of the sylvian, the posterior lateral and posterior part of the middle lateral gyrus. In a few experiments ether anesthesia was substituted for nembutal, and the depth of the anesthesia was varied from a light stage to respiratory arrest without changing the gyri giving responses to the lobulus paramedianus. This, however, does not prove that connections might not be established by less direct route from other areas which in the conditions of our experiments failed to give responses. The connections demonstrated here may represent only the most direct connections and not the total possibilities.

The responses were the largest and most readily obtained in the lobulus ansiformis, lobulus paramedianus and paraflocculus. Culmen, folium and tuber vermis and pyramis were somewhat less apt to show action potentials, but even here the responses occurred from widely scattered parts of the cerebral cortex. A comparison was made of the responses in the various cerebellar lobules when points within topographical subdivisions of the cat's cortex were stimulated. Face, arm and leg areas either were determined by stimulation with low voltage alternating current, or points were selected on the basis of Adrian's (1940, 1941) map of the somatic sensory areas. The cerebellar lobes which showed action potentials were the same regardless of which topographical area was stimulated, and no difference in the threshold or in the amplitude of the responses could be detected.

Although the sign of the potential when a "monopolar" lead was placed on the surface of the cerebellum was usually surface positive, there were frequently important surface negative elements in the potential. It was not possible to predict what the sign or form of the potential might be. Sometimes a shift of the stimulation electrode to a new point might reverse completely the sign of all components of the potential recorded from an identical cerebellar lead point without disturbing the time sequence of any part of

the potential as measured from the shock artifact. The form of the potential frequently was different for different stimulation points with identical lead points and even from a series of stimuli applied to the same point in rapid succession. These differences were naturally accentuated when leading with bipolar electrodes, but might be present also with single active electrodes. A stimulation by needle electrodes of the subcortical white matter (Fig. 1D) elicited this also.

The response can be inhibited for relatively long periods by a previous shock at the same cerebrocortical point. This inhibitory effect may be present

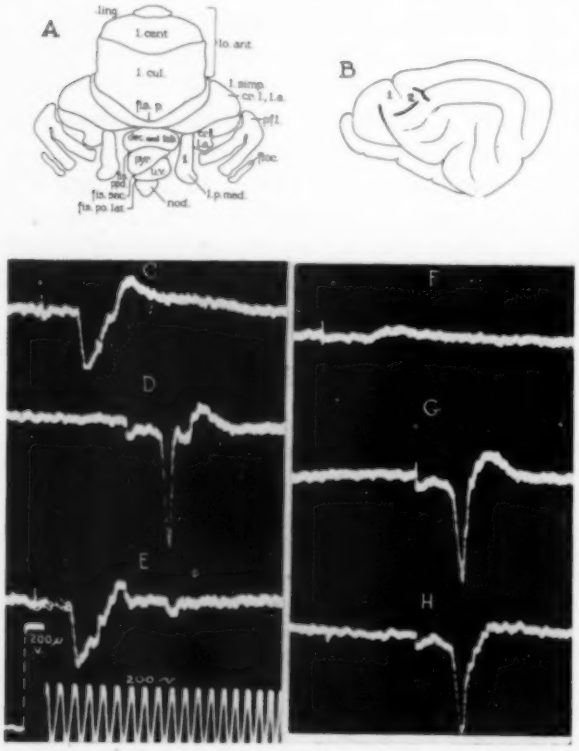


FIG. 2. A. Schema of the cat's cerebellum. Point 1 shows lead on right lobulus paramedianus used for records C–H. B. Left cerebral hemisphere of the cat. (1) Stimulus point for conditioning shock, hind limb area 4. (2) Stimulus for testing shock, forelimb area 4. C. Conditioning response alone. Stimulus strength 2× threshold. D. Testing shock alone. Stimulus strength 2× threshold. E. Both. Note almost complete elimination of testing response at 32 msec. interval. F. Conditioning alone after local application of 5 per cent cocaine at point 1 for 5 minutes. Stimulus strength as in C. Note elimination of response. G. Test alone. Stimulus strength as in D. Comparison with D shows it to be unaffected by cocaine. H. Both. Note almost complete absence of effect on testing shock.

In these and all subsequent records a deflection down is a surface positive potential.

for as long as a second. Conversely the size of the response frequently increased from shock to shock, even though successive shock were delivered about 4 sec. apart. A detailed investigation of factors for facilitation and inhibition has not been made, but it is obvious that the corticopontocerebellar system is subject to facilitation and inhibition as are other types of cortical activity. Stimuli applied to widely separated cortical points in separate topographical subdivisions can completely abolish each other for many milleseconds (Fig. 2C–E). One cannot be certain from the data at hand that this is not due to intracortical connections. By the use of cocaine at the site of stimulation it was shown that current spread was not responsible for the conditioning effect (Fig. 2F–H).

Monkey. The cerebellar divisions from which responses were obtained in *Macaca mulatta* correspond closely to those in the cat. The responses differed only in that those on the culmen were on the whole larger than in the cat, and in one experiment significant potentials were present in the uvula. As in the cat, there were responses also in lobulus simplex, lobulus ansiformis, lobulus paramedianus, paraflocculus, folium and tuber vermis and pyramis.

In a few monkeys the sciatic nerve was stimulated alternately with the stimulation of the cerebral cortex. In the monkey there is some spread of the cerebrocortical projection into cerebellar subdivisions which in the cat exhibit few or no responses following stimulation of the cerebral cortex. As a corollary the spinocerebellar connections are less widespread in the monkey than in the cat. In the cat it would appear that the whole of the culmen is supplied with spinocerebellar connections (Dow, 1939), while in the monkey responses to sciatic nerve stimulation are restricted to the medial parts of the lobe (Fig. 3C). The latency of the response from sciatic stimulation varied from 18 to 25 msec., as compared to 8 to 13 in the cat (Dow, 1939). It was usually a surface positive deflection.

In the monkey, as in the cat, responses were

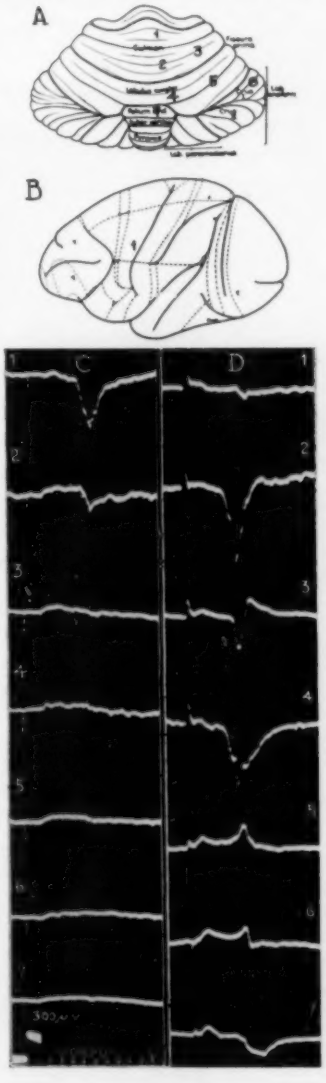
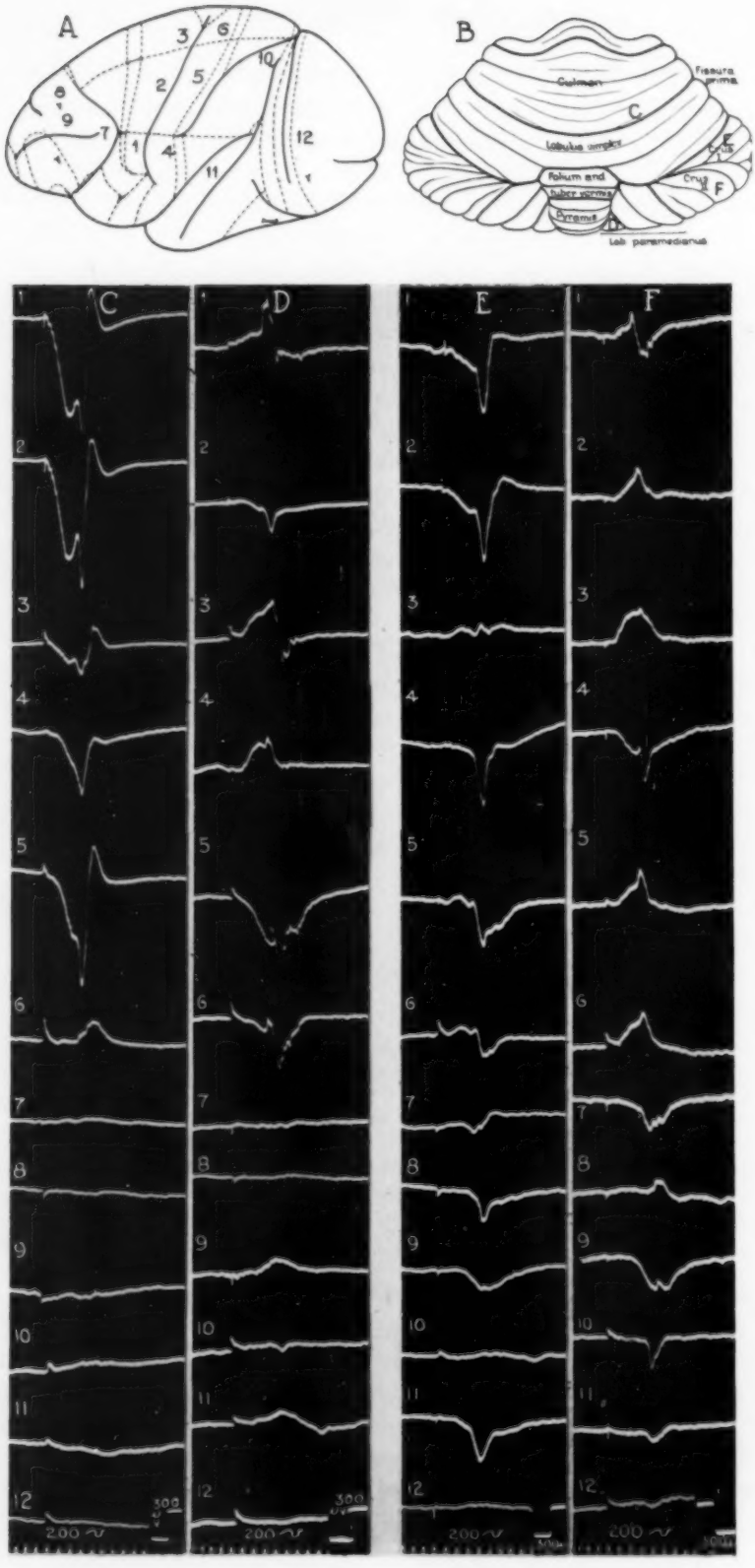


FIG. 3. A. Diagram of the anterior surface of the cerebellum of *Macaca mulatta*. Points 1 to 7 indicate the lead points for the respective records in C and D. B. Diagram of the left cerebral hemisphere of the *Macaca mulatta*. (1) Indicates the point of stimulation for the responses shown in D 1–7. C. Responses from single shock electrical stimulation of the homolateral (right) sciatic nerve. 1–7 indicate records at respective lead points as in diagram A. Note response limited to midline of culmen. D. Response from stimulation at point in arm area 4 on contralateral cortex. Note response in posterior lobe and throughout culmen, particularly in upper and lateral part.

in widely separated parts of the cerebellum from stimulation of a single point on the opposite cerebral hemisphere (Fig. 3D). Conversely single lead points on the cerebellum showed potentials when the stimulus was applied over a wide area of the cerebral cortex (Fig. 4). Homolateral responses were obtained also, but they were of lower amplitude and most marked near the midline. Responses from stimulation of the most widely scattered cerebral areas were elicited when leading from the lobulus ansiformis (Fig. 4E and F, 5F).


A
B
Culmen
Fissura prima
Lobulus simplex
Folium and tuber vermis
Pyramis
Lob paramedianus
C
D
E
F
200
300

Responses when leading from the lobulus ansiformis were present following stimulation of Brodmann's areas 8, 9 and 10, areas 4 and 6, the entire parietal lobe, area 22 and occasionally areas 21, 18 and 19. In no instance were responses obtained from a stimulus at the rostral part of area 9 or in the area striata (17). The extreme frontal and occipital poles and the inferior temporal gyrus (area 20) were not explored, nor were the orbital or medial surfaces of the cortex. The areas consistently lowest in threshold responses were area 4-s and area 6 (Fig. 6).

Responses from leads on the lateral superior part of the culmen were present when the stimuli were applied to a much more restricted part of the cerebral hemisphere (Fig. 4C and 5D). In this case no action potentials were found when areas 8, 9 or 10 were stimulated. Following stimulation of areas 4, 4-s and 6, responses were elicited but those of lowest threshold were as a rule in areas 4 (Fig. 6), and those from the parietal lobe were best seen when the stimulus was near the central fissure. There were no responses from stimulation of areas 22, 21, 19, 18 or 17, and only two out of eight points stimulated in area 7 gave slight responses. Lead points lower and more medial on the culmen showed action potentials following stimulation of the same areas, but the responses were of much lower amplitude and had higher thresholds as a rule (Fig. 3D, and 5B).

Responses in the pyramis and the lobulus paramedianus were most consistent following stimulation of the precentral and postcentral gyri (Fig. 5C, E). Leads on the paramedianus and pyramis, however, showed occasional responses following stimulation of points rostral to the arcuate sulcus in the frontal association areas and in the temporal and posterior parietal lobes. These cerebellar lobules appear to occupy an intermediate position between the culmen and the lobulus ansiformis, in respect to the extent of the cortical areas having corticopontocerebellar connections demonstrable by this method.

FIG. 4. A. Diagram of the cerebral hemisphere of *Macaca mulatta*. Points 1–12 indicate stimulation points for their respective records in C, D, E and F. Records in C and D and in E and F are simultaneous potentials from the same stimulus. B. Diagram of the anterior surface of the cerebellum of *Macaca mulatta*. Points C, D, E, and F indicate lead points for records shown at C, D, E and F respectively. C. Records with a monopolar lead on the upper, lateral part of the culmen. Numbers refer to the stimulation points in diagram A. Stimulus strength 140 V. peak voltage, 0.3 msec. duration for all responses. Note absence of responses from points outside areas 4–6 and postcentral gyrus. Note the low amplitude of response from a stimulus in leg areas. D. Records with monopolar lead on the lobulus paramedianus. Numbers refer to the stimulation points shown in diagram A. Each record taken simultaneously with corresponding records in C and represent the response to the same stimulus at the two points for comparison. Note the similarity to C in the cytoarchitectonic areas giving responses but with relatively greater amplitude in the responses from leg areas. E. Lead in Crus I lobulus ansiformis. Numbers refer to the stimulation points in diagram A. Note responses 7–11 from stimuli in widespread cortical zones. F. Lead at Crus II lobulus ansiformis. Numbers refer to stimulation points in diagram A. Each record simultaneously with the record of like number in E represents the response to an identical stimulus at the two different lead points. Note absence of response in all parts of the cerebellum from point 12 in Brodman's area 17.

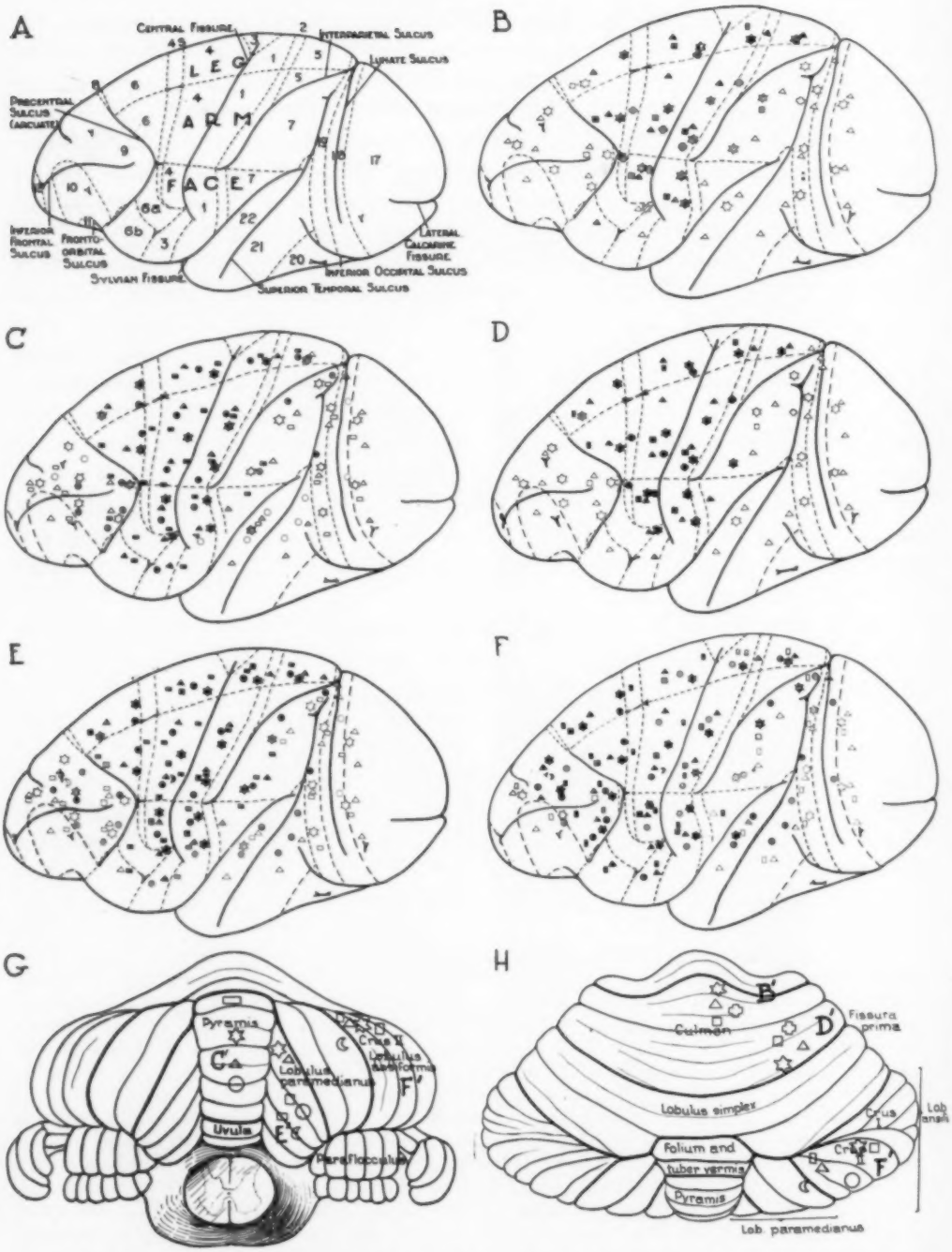


FIG. 5. A. Master diagram of the cerebral cortex of the *Macaca mulatta* showing both cyto-architectonic areas and topographical subdivisions as delineated by Dusser de Barenne, Garol and McCulloch (1941). B. Showing diagrammatically the responses at the medial lower parts of the culmen in 4 experiments. Geometric figures shown in H (B′) are lead points from which the responses were recorded. The stimulation points corresponding

Analysis of results simply on the basis of the presence or absence of responses with strong stimulation, without regard to the amplitude of the response or the threshold strength of the stimulation necessary to produce it, shows no other differences between the lobes of the cerebellum in respect to their corticopontocerebellar connections than we have already indicated. However, by giving each response recorded a rough quantitative rating on the basis of the amplitude of the response or its threshold, certain differences have come to light. These differences in threshold and in the amplitude of the response to stimuli of like strength may indicate differences in the relative richness of the projection from various parts of the cerebral cortex to various parts of the cerebellum within the pontine projection area. Figure 6 graphically depicts these differences. One may see that the projection from the face area is greatest in culmen and lobulus ansiformis Crus II, while that from the leg area is most marked in lobulus paramedianus and to a lesser degree in pyramis. It should be emphasized, however, that responses from low threshold stimulation in any topographical subdivision were obtained in all the lobes. No significant differences in the threshold of responses in Crus I and Crus II, lobulus ansiformis could be detected in any cortical subdivision, either topographical or cyto-architectonic. The percentage of low threshold responses from face and arm subdivisions was slightly higher in Crus I than in Crus II, while that from stimuli in the leg areas was slightly lower. In both lobules the percentage of low threshold responses in face areas was more than double that for either arm or leg areas.

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to each of the four lead points are represented by the same geometric figure. Each response or group of responses from that particular lead point was given a rough quantitative rating. No response is shown as a blank, + by a single cross hatching, + + by double cross hatching, + + + responses (*i.e.* those of lowest threshold or highest amplitude or both) by a solid block. Note the rarity of low threshold, high amplitude responses and the restriction of almost all responses to the pre- and postcentral gyri. C. Diagrammatic representation of responses at pyramis (see G) (C′). Note relatively larger proportion of + + + responses in leg area as compared to D and F. D. Same for upper lateral part of culmen (see H) (D′). Note similar total response distribution to B, but with a large proportion of + + + responses in pre- and postcentral gyri, particularly in face area. E. Same for 6 experiments with leads on lobulus paramedianus, lower part (see G) (E′). Note similarity to pyramis in the distribution of all responses, and of + + + responses. F. Same for 5 experiments with leads in Crus II lobulus ansiformis and one lead on border between lobulus paramedianus and Crus II (see G and H) (F′). Note more widespread area of responses with significant numbers of + + and + + + responses in frontal association areas, and in posterior, parietal, temporal, and peristriate areas. G. Posterior view of the cerebellum of *Macaca mulatta*. Each geometric figure indicates a lead point for a particular experiment. B′ to F′ indicates lead points for the stimulation points shown in the respective diagrams, B to F. H. Some as G for the anterior view of the cerebellum in *Macaca mulatta*.

It is recognized that it is impossible to place points accurately within the finer cyto-architectonic subdivisions, and errors will arise in transposing points to a master diagram such as this. However, many points in area 4 were verified at the time of the experiment by low voltage A.C. stimulation. Some points in area 4-s were identified by observing suppression effects as described by Dusser de Barenne and associates, and it is felt that, so far as the major subdivisions and gyri are concerned, the points represent the actual site of stimulation.

Figure 7C, D shows a comparison between two points within the lobulus paramedianus as originally outlined by Bolk (1906). Responses of rather high amplitude were observed at the upper point from stimulation in area 9 (Fig. 7D4). Such a response was not seen in the lower folia in this experiment (Fig. 7C4) nor in other experiments in which the lower folia of lobulus paramedianus were led from. The responses from the upper folia of lobulus paramedianus were not typical of those from lobulus ansiformis, since these of lowest threshold and highest amplitude result from stimulation of area 4 (Fig. 7D1) and not area 6 (Fig. 7D3), while the reverse is characteristic

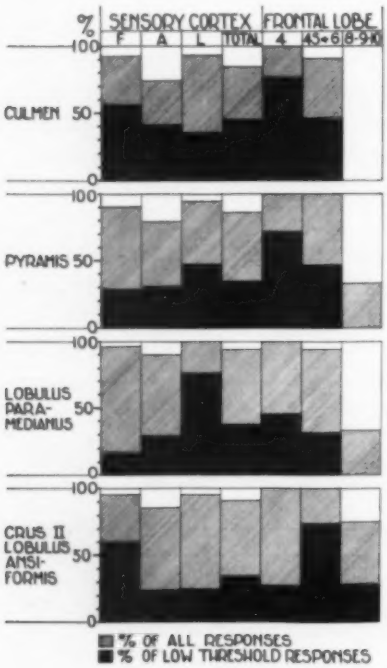

FIG. 6. Chart showing the presence and relative strength of responses from stimulation of topographical and cyto-architectonic subdivisions of the cerebral cortex when leading from culmen, pyramis, lobulus paramedianus and Crus II lobulus ansiformis. Data given in percentage of the total points stimulated in all the experiments. Note very little differences in total responses (cross hatching) except in areas 8–9–10. The solid black shows percentage of + + + responses (responses of low threshold and high amplitude). When the percentages of low threshold or high amplitude responses are compared, differences are detected. Note differences between lobulus paramedianus and lateral part of the culmen in respect to topographical subdivisions and the greater percentage of + + + responses from face than either arm or leg in the Crus II lobulus ansiformis. In comparing the various cyto-architectonic areas of the frontal lobe, the greater percentage of + + + responses in vermian lobes from stimuli in area 4, while stimuli in area 6 and the frontal association area show the greatest percentage of + + + responses in the lobulus ansiformis.

of lobulus ansiformis. There are apparently greater differences in the responses from two points within lobulus paramedianus than appear to exist between Crus I and Crus II of lobulus ansiformis, or between culmen and lobulus simplex.

The sign of cerebellar action potentials resulting from stimulation of the contralateral cerebral cortex in the monkey was not predictable. The responses were usually surface positive but might be negative or diphasic or exceedingly complex. At times a shift of the stimulation point would change completely the form and sign of the cerebellar action potential (Fig. 4F4, 5), and at other times might completely reverse the sign of the potential without altering the time course of any components. Shifting a stimulus might fail to alter the response at one lead point in any particular, while the responses from the identical stimuli were different at another lead point (Fig. 4C1, 2; D1, 2). Thè responses, as in the cat, seemed to separate them-

selves into those of short latency and those of somewhat longer latency. Responses of latencies approaching those of 200 msec. recorded by Curtis (1940) in the cat were not seen in the monkey, but no particular effort was made to search for them, and they may well by present in the monkey under certain conditions. The commonest response, the one most frequently elicited at threshold strength stimuli and, with rare exceptions the only type when one stimulates in the areas 8–9–10, has its peak from 18 to 30 msec. after the shock artifact. In general the greater the voltage of the action potential the shorter the latency of the response within these limits. Also it was frequently possible to demonstrate reduction of the latency of the response by several milliseconds on gradually increasing the strength of the stimulus. The other response was of much shorter latency. It too might be negative or positive in sign though most frequently the latter. It usually appeared when the stimulus was

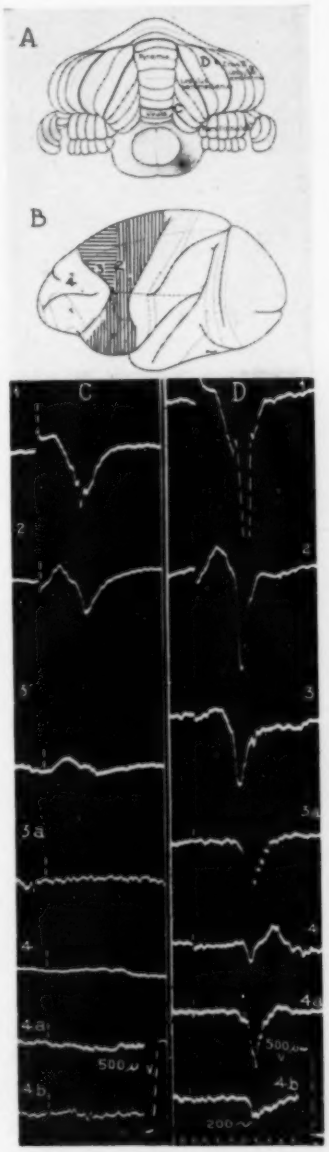

FIG. 7. A. Diagram of the posterior surface of the cerebellum of *Macaca mulatta*. Lead points for records C and D, at points C and D respectively. Note that both lie within the lobulus paramedianus as delineated by Bolk (1906). B. Diagram of the cerebral hemisphere of *Macaca mulatta*. Points 1, 2, 3 and 4 indicate sites of stimulation for records shown in C and D. Vertical cross hatching indicates area ablated before records 3a and 4a were taken. Horizontal cross hatching indicates area subsequently ablated but before record 4b was taken. Point 2 was identified as being in area 4-s by obtaining a suppression effect of Dusser de Barenne and McCulloch (1941). C. Action potentials from lead points C in lower part of lobulus paramedianus. Records 1, 2, 3 and 4 from stimulation of the respective points as shown in diagram B. 3a and 4a records from stimulation of points 3 and 4 after ablation of area 4. 4b record from stimulation at point 4 after ablation of areas 4 and 6. D. Records of action potentials recorded from a lead at point D, taken simultaneously with those shown in C. Note differences in responses from the same stimulus, particularly from stimulation at points 3 and 4 in areas 6 and 9 respectively. Note in 3a and 4a and b the continual presence of potentials after ablations as indicated in B have been made.

applied in the precentral or postcentral gyri and usually it had a higher threshold than the later appearing potential. At strong shock strengths both might be seen (Fig. 4C1, 2). The two might be the same sign, Fig. 4C2, or the reverse of one another, D3. The slope of the rising phase of these early potential is usually less steep than the later one. Its peak is frequently not

identifiable. This potential might begin as early as 3 msec. after the shock artifact. It can be picked up with bipolar needle electrodes placed on the surface of the cerebellum and undoubtedly is produced by nervous tissue in the cerebellum at the lead site. Which part of the whole potential complex is due to the afferent fibers to the folium and which is due to the activity of neurons in the cerebellar cortex is not clear. The reason for the responses of these two latencies is likewise unknown. It is presumed that both represent activity produced by corticopontocerebellar connections. Stimulation with needles in the white matter of the cerebral hemispheres did not materially affect the latencies of the responses, and both the early and late type could be seen (Fig. 1D). It was noted that when successive identical stimuli were given at the same stimulation point and the same lead point the early response was usually identical from stimulus to stimulus while the response whose peak occurred from 18 to 30 msec. was much more variable in its sign and form from stimulus to stimulus.

In one experiment responses were studied following the successive ablation of area 4 and then area 6. The responses from stimulation of area 6 are unchanged by the ablation of area 4 (Fig. 7D3a) and those from the post-central gyrus and the frontal association areas were still obtained subsequent to ablation of area 6 (Fig. 7D4a, b).

Discussion

Attempts to find any exclusive point-to-point relationship between subdivisions of the cerebral cortex and single lobules of the cerebellar cortex were unsuccessful. This was particularly true in the cat, and is in entire agreement with the observations of Curtis (1940) and in harmony with anatomical investigations of Sunderland (1940). In the monkey there seems to exist a predominance of representation from certain areas to certain cerebellar lobes. The cyto-architectonic subdivisions of the frontal lobes particularly exhibited such tendencies. Here area 4 has its richest projection to the vermian and paravermian lobes, while area 4-s and area 6 send their richest projection to lobulus ansiformis, and the frontal association areas are almost exclusively connected with the lateral lobes. Areas 7, 18 and 22 also are predominantly connected with lobulus ansiformis, while the post-central gyrus is relatively greater in its connections to culmen, pyramis and lobulus paramedianus. It is useless to speculate at this time on possibilities of the functional importance of these differences in afferent connections between vermis and hemisphere. Comparison between the topographical subdivisions of face, arm and leg areas of the so-called "sensory cortex" as mapped out by Dusser de Barenne and his associates (1938, 1941) shows a much less definite tendency for predominance to a particular lobe. However, by the use of a rough quantitative estimate of the responses by an evaluation of their threshold and the amplitude of the response, it does seem that the more rostral vermian and paravermian lobes find a larger face representation, while the more caudal lobules have the richest supply from the leg areas. This, how-

ever, is only a quantitative tendency and not in any sense an exclusive representation. Little difference could be detected between Crus I and Crus II of lobulus ansiformis, even by this rough quantitative estimate. The entire lobulus ansiformis apparently received a heavier projection from the face subdivisions than from either arm or leg subdivisions. The afferent corticocerebellar connections to Crus II lobulus ansiformis as determined by this method do not conform with the comparative anatomical data of Bolk (1906) and of Larsell and von Berthelsdorf (1941), which suggested a relationship between the Crus II and the cerebellar control of the lower extremity. This work does not disprove any such topographical relationship, but the afferent connections certainly do not suggest such topographical localization of function in the cerebellum. Furthermore, the fact that a cerebellar action potential produced by stimulation of a point in the forelimb area can be completely inhibited by a previous stimulation of a point in the hind limb area suggests that at some point there these two cerebellar projections share the same neurons. It is felt that this is probably in the pons or in the cerebellar cortex, but the effect might be the result of intracerebrocortical connections. This evidence for a common pathway between two topographically distinct areas of the cerebral cortex and one cerebellar lobe points against a topographical localization within the part of the cerebellum which is dominated by corticopontine connections.

The presence of action potentials on stimulation of the postcentral gyrus and frontal association areas after the ablation of areas 4 and 6 in an acute experiment shows that these lobes have their own corticopontine fibers, and the responses from the various cortical points are probably not dependent upon intracerebrocortical relays. A complete discussion of previously described anatomical pathways which might carry these connections is not given here, as recent contributions are covered in a current review (Dow, 1942). One might say that the finding of action potentials in the cerbellum from the stimulation of area 9 after ablation of areas 4 and 6 supports the contention of Mettler (1935) and Levin (1936) that some frontopontine fibers originate in areas rostral to area 6. One should call attention to the finding that parietal projections were much more widespread and apparently more important than temporopontine connections. Furthermore, the postcentral gyrus was apparently the richest zone of all the parietal lobe projection areas.

The lack of uniformity of the sign and form of the potentials is not unexpected in view of the great complexity of the cerebellar folial pattern, with its numerous fissures and sulci. The importance of the relation of the lead electrode to cerebellar fissures in the sign of the potentials resulting from stimulation of afferent fibers has already been reported in the case of the stimulation of the olivocerebellar fibers (Dow, 1939). It seems doubtful, even though responses from stimulation of a single cortical point are picked up over the whole corticocerebellar projection area, that all pontocerebellar fibers are activated by any one cortical stimulation, no matter how strong.

The cerebellar action potentials following stimulation in the pons (Dow, 1939) were much more uniform, but in this case probably a larger percentage of the pontocerebellar fibers were activated with each stimulus, no matter in which part of the pons the needles were placed.

Differences in the corticocerebellar and spinocerebellar connections in the cat and monkey suggest that in addition to an increase in the monkey in the size of the hemispherian lobes which have purely corticocerebellar connections, there is an invasion by corticopontocerebellar fibers of vermian lobes, which in the cat have few or none of these fibers. Coincidentally with the increase in the corticocerebellar connections in these border-line lobules there is a decrease in the spinocerebellar connections. This supports Winkler's (1923) contention that the neocerebellar connections grow into pre-existing cerebellar lobes and that the "neocerebellum" is not a separate group of cerebellar lobules superimposed upon other lobules which are exclusively paleocerebellar. These species differences in anatomical connections may also explain certain species differences in the effects of ablation of subdivisions of the cerebellum. It should make one guard against too ready transfer of data from experiments in one animal to those of another, particularly to man, where there is undoubtedly even more of an overgrowth of pontine connections than here demonstrated in the monkey.

There is no indication from this study of afferent connections that all the individual cerebellar lobules delineated by Bolk (1906) within the corticopontocerebellar projection area have functional significance. Corticocerebellar connections are not limited by these boundaries, and in certain instances there may be important differences in afferent connections within one of these subdivisions. For example, in the monkey the lateral and medial parts of the culmen are totally different in respect to spinocerebellar connections, and the upper and lower parts of the lobulus paramedianus in respect to the connections from the frontal association areas. It would appear from their afferent connections that many of these lobules defined by Bolk, convenient as they are for descriptive purposes, have little or no functional significance. A study of efferent connections may be of more significance in this regard, however, and these observations on afferent connections do not disprove a localization of function along the lines advocated by Bolk.

Summary

Single shock electrical stimulation of the cerebral cortex results in action potentials in the cerebellum in the cat and monkey. These responses recorded oscillographically are most marked contralaterally, but homolateral responses are also present. The stimulation of a single cortical point with a well localized stimulus may result in action potentials in all the cerebellar lobes which receive pontocerebellar connections.

Action potentials, in response to stimulation of the cerebral cortex in the cat, were observed in culmen, lobulus simplex, lobulus ansiformis, declive and tuber vermis, paraflocculus and pyramis. In the monkey, in addition to

these lobules, responses were present in the uvula. Border-line zones between the pontine and spinal subdivisions of the cerebellum in the cat were dominated by corticopontocerebellar connections in the monkey. It appeared from the restricted zone of spinocerebellar connections in the monkey, as compared to the cat, that this growth of pontine connections was to some degree at the expense of spinocerebellar connections.

In the cat cerebellar action potentials were elicited most consistently following stimulation of the sigmoid, coronal, anterior ectosylvian and anterior parts of the lateral, suprasylvian and middle ectosylvian gyri. There were isolated responses in occasional experiments when the posterior ectosylvian, posterior suprasylvian and posterior part of the middle ectosylvian gyri were stimulated. No action potentials were seen following stimulation of the sylvian and remaining divisions of the lateral or marginal gyri. No other areas of the cat's brain were explored in these experiments. Among the areas stimulated in the cat, no topographical or cyto-architectonic subdivision appeared to be represented exclusively or even predominantly in any cerebellar lobe.

In the monkey the most widespread cerebellar action potentials were evoked when areas 4 and 6 and the postcentral gyrus were stimulated. In addition, the stimulation of areas 8, 9 and 10 in the frontal lobe, areas 7, 18, 19, 21 and 22 in the parietal, occipital and temporal lobes, produced action potentials in the cerebellum, particularly in lateral and posterior lobules such as the lobus ansiformis. Conversely the projection, as judged by the amplitude and threshold of the responses, was relatively greater from the pre- and postcentral gyri to the vermian and paravermian lobules.

Differences in the amplitude of the action potentials and in strength of the threshold stimulus necessary to produce them were less marked when the topographical subdivisions of the cerebral cortex were compared. By comparing the percentage of low threshold points in the face, arm and leg subdivisions of the "sensory" cortex, as delineated by Dusser de Barenne and his associates, when leading from some of the cerebellar lobes, certain differences were observed which may indicate a slight preponderance of connections to certain lobes from these topographical subdivisions. Among the vermian and paravermian lobes there was a tendency for the face area to show the greatest percentage of low threshold responses in the more rostral lobes, such as culmen, while the projection from the leg zone was judged heaviest in the caudal lobules, such as lobulus paramedianus and pyramis. The arm area was intermediate between these two extremes. No differences between topographical areas could be detected if only the presence or absence of response was observed and some low threshold points to culmen were noted in the leg zone and vice versa. No significant difference in threshold or amplitude of the responses between Crus I and Crus II of lobulus ansiformis could be demonstrated when either cyto-architectonic or topographical subdivisions were compared. They both showed the largest percentage of low threshold responses from stimulation of the face areas. There was no evi-

dence from this study of afferent connections that the cerebellar control of the lower extremity rested in Crus II of the lobulus ansiformis.

The author wishes to express his thanks to Mr. Fred Claussen for technical assistance.

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THE EFFECTS OF POLARIZATION ON NERVE ACTION POTENTIALS*

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THE PROLONGED potential changes accompanying activity in polarized nerve are commonly regarded as changes in the polarization or electronic currents due to changed resistance or polarizability of the membrane (25, pp. 300–301; 28, p. 16; 10, pp. 812–814; 23, p. 715).

This explanation applied to the shorter potential changes (extrapolar decrease of polarization current and intrapolar increase of polarizing current during activity) was contested by Hermann in the early days of nerve physiology, who formulated his increment-decrement law as an alternative explanation of the experimental facts (17, 18, 19). According to this law, the modification of the polarization and polarizing currents by activity is determined by the size of the action potential wave, and this in turn is determined by the degree of polarization of the reactive membrane, not by its polarizability. The changes embodied in the increment-decrement law, increase of spike height at the anode of a constant current and decrease at the cathode, have been demonstrated beyond question in the action potential of frog A fibers by cathode ray oscillograph experiments (3, 23, 11), and the "law" is now the generally accepted interpretation of the shorter potential changes in active polarized nerves.

The inability of the "law" to explain the longer changes on the basis of the action potential as known sixty years ago was recognized by Hermann himself within ten years after his first announcement of it, and he then accepted changed polarizability as an adjunct necessary for the complete explanation of the experimental observations (20). In the light of present-day knowledge of the slower components of the action-potential, the increment-decrement law again becomes an adequate formulation of the facts, provided the negative after-potential resembles the spike in its changes of size under polarization.

In the experiments reported here, the effects of polarization on the amplitude of both the spike and the after-potentials of mammalian and amphibian A and C fibers were investigated. The effects of continuous polarization on nerve activity have been studied heretofore chiefly on frog A fibers, and the results of some of these earlier studies are quoted in this paper. Certain unmyelinated nerves (mantle nerves of molluscs, 5, 29; pike olfactory, 7) have also been investigated when polarized, and have been found to obey the

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increment-decrement law in a general way. Amphibian and mammalian C fibers have apparently not been investigated from this point of view, and the only available information on mammalian A fibers comes from a string galvanometer experiment of Verzár's (28, p. 11).

METHOD

For mammalian A fibers, the saphenous nerve of the cat was used; for mammalian C fibers, the hypogastric, vagus or other autonomic nerve of the cat. The sciatic nerve of Rana pipiens provided amphibian A fibers, the splanchnic nerve of Rana catesbiana amphibian C fibers. The action potentials of an isolated nerve mounted in a moist chamber were observed and photographed on a cathode ray oscillograph after amplification by a direct-coupled amplifier; the sweep circuit and stimuli were synchronized electrically (21). The stimuli were induction shocks from thyratron stimulators for A fibers, and induction shocks or more usually constant currents for C fibers (3–4 V., 3–5 msec. for amphibian; 2–6 V., 1–3 msec. for mammalian). The stimulus in each case was made strong enough to evoke a response from all the fibers, A or C as the case might be.

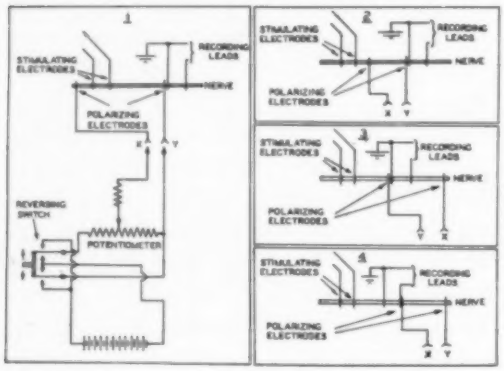

FIG. 1. Polarizing circuit and electrode arrangements. See text.

The polarizing current was applied through polarizable or nonpolarizable electrode; the pole whose effect was to be studied (the adjacent pole) was usually directly in contact with the ground lead to the amplifier, but occasionally a millimeter or so away from it on the nerve; the remote pole was 4–35 mm. (usually between 10 and 20 mm.) away. Figure 1, 1, shows the most satisfactory order of electrodes on the nerve for eliminating all possibility of unintentionally influencing the record by the effects of the remote pole; this arrangement was usually adopted with A fibers, but frequently produced unmanageably large shock artefacts with the constant current stimulation used for C fibers. Diagram 4 shows another arrangement for complete elimination of the effects from the remote pole; its disadvantage lies in interference with the early elements of the polarized (second) phase of the response by the late elements of the first phase. Diagram 3 shows a convenient but treacherous arrangement for polarization; when the remote pole is the anode, its effects are transmitted through considerable distances to the grid lead, and this introduces serious complications in the record if the response is at all diphasic. With the arrangement shown in diagram 2, the effects of the remote pole may interfere with the electrical stimulation of the nerve or the transmission of the response to the adjacent pole; with strong polarization, for instance, cathodal block at the remote pole may account for what seems to be anodal depression at the adjacent pole. Except when the polarizing current was applied as in 4, the nerve was usually crushed or heated at an appropriate point in the attempt – notably unsuccessful in the case of C fibers—to make its response monophasic. With proper precautions in interpretation, all of the above arrangements of course give comparable results.

The polarization circuit was closed by hand, and time allowed (a few seconds up to 5 min.) for the transient effects of the polarization to pass off. From the applied voltage as read on a calibrated slide wire, the fixed resistance in the circuit, and the resistance (40,000 to 200,000 Ω) of the polarized stretch of nerve measured at the end of the experiment, the voltage drop across the nerve stretch and the current could be calculated. The voltage drop so calculated was usually between 0.1 and 5 V., the current between 1 and 50 μA.; the numerical values are however of little significance, since the apparent magnitude of polarization necessary to produce similar effects in different nerves of the same kind

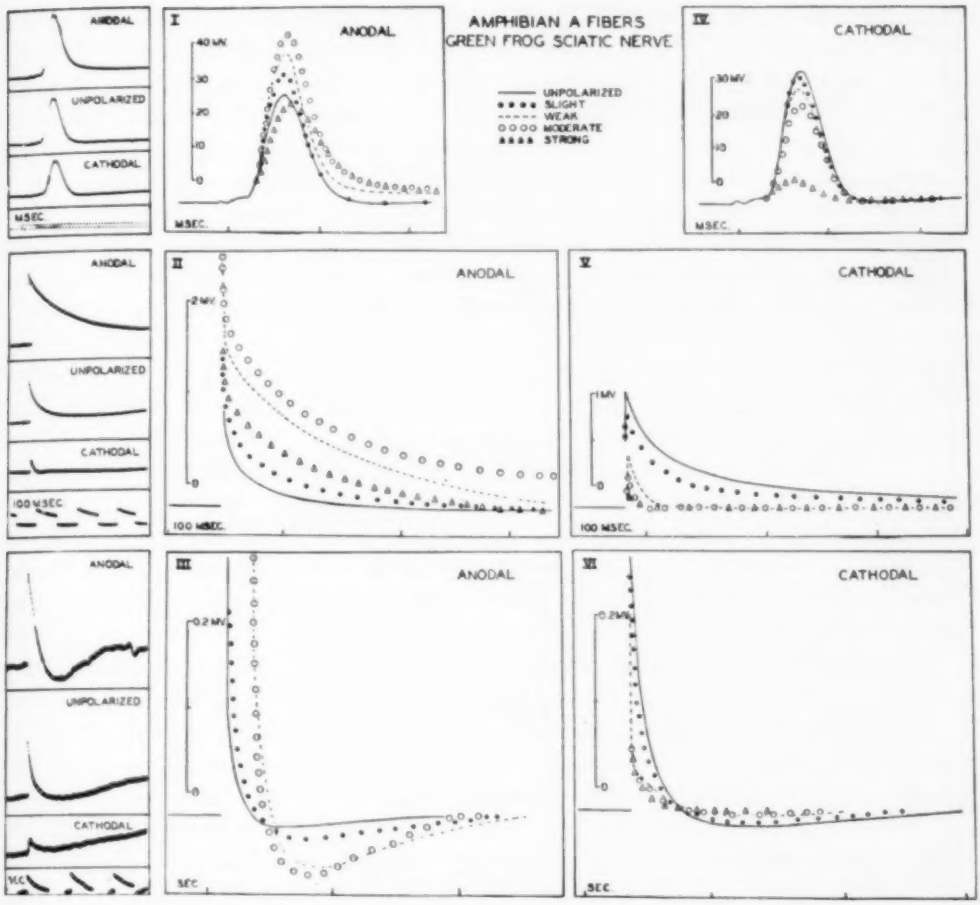

FIG. 2. Effects of polarization on amphibian A fiber action potentials. Each diagram (I–VI) consists of superimposed tracings of records taken at the indicated amplification and speed with various strengths of polarization. The effects at each pole are shown by 3 diagrams at 3 different amplifications and speeds. Actual records at zero and moderate polarization reproduced at left of each pair of diagrams. Slow tracings corrected for drift of base line seen in records. Electrode arrangement 1; interpolar distance, 28.5 mm.; interlead distance, 8 mm.; conduction distance, 13 mm. 7/5/39.

varies widely, presumably due to variation in such factors as the amount of adherent moisture and connective tissue.

A fairly rapid drift of potential frequently persisted for so long in nerves that had been polarized that it was impossible to postpone further work until its disappearance, and it therefore appears in the slow records reproduced.

Within the limits indicated below, all the effects to be described are promptly reversible.

RESULTS

A fibers. The decrease of the spike at the cathode and its increase at the anode of a constant current, already well established for the response of frog A fibers (3, 23, 11) and illustrated in Fig. 2, I and IV, is duplicated in

the response of cat A fibers (Fig. 3, I and IV). With either frog or cat nerve the maximal height of spike observed under anodal polarization is usually 20–40 per cent greater than the height of the spike produced by the unpolarized nerve, but the maximal increase varies from 10 per cent (or occasionally even less) to 100 per cent with individual nerves. The potential drop across the nerve for the maximal spike height is in the neighborhood of 0.5–1 V., the current 10 μA. or less. As the polarization is strengthened above this, the spike at the anode falls below its maximal height, then below normal height, and finally disappears. (Polarization of about the strength resulting in maximal spike increase at the anode will be designated as "moderate"; polarization of significantly less strength, "weak"; of still less strength, "slight." Polarization stronger than moderate will be designated as "strong," "stronger" or "extreme," according to its strength. Between any two successive steps, the voltage was usually increased $1\frac{1}{2}$–$2\frac{1}{2}$ times.)

The fall of spike height observed at the anode with strong polarization is attributed to blocking of the response of some fibers in the nerve, since Erlanger and Blair observed with single fibers an increase at the anode up to the blocking strength of polarization, *i.e.*, no decrease preliminary to block. Their published results (11, Fig. 2, 3, 7) show an increase of somewhat over 100 per cent before block; the maximal increase of the nerve spike is thus notably less than that of the single fiber spike, probably because some of the constituent fibers of the nerve are blocked by a strength of polarization too low to induce the maximum spike in others. Greater temporal dispersion due to slower conduction at the anode (3, p. 639) may also be a factor in reducing the observed increase of the nerve spike.

With cathodal polarization of moderate strength, the spike is 40–80 per cent of its normal height, and it is greater or less than this according to the strength of the polarization. When with sufficiently strong polarization the height of the spike falls below normal at the anode, it is always still lower at the cathode (Fig. 2, I, IV; Fig. 3, I, IV), in agreement with the above interpretation of the fall at the anode.

It is known from the earlier work of others that with the increased spike height at the anode goes an increased spike duration (3, p. 645; 23, p. 716; 11); such prolongation was observed in these experiments (Fig. 2, I; Fig. 3, I), but was not investigated further.

Like the spike, the negative after-potential of A fiber responses is increased at the anode and decreased at the cathode. With both amphibian and mammalian A fibers, the increase of the negative after-potential at the anode is relatively greater than that of the spike; the after-potential may indeed be increased to several times its original size by appropriate polarization (Fig. 2, II; Fig. 3, II). The increase in amplitude of the negative after-potential with increase in strength of polarization ceases only when the polarization is strong enough to result in decreased height of spike at the anode. With weak polarization the anodally increased amplitude of the negative after-potential is usually accompanied by increased duration, especially in

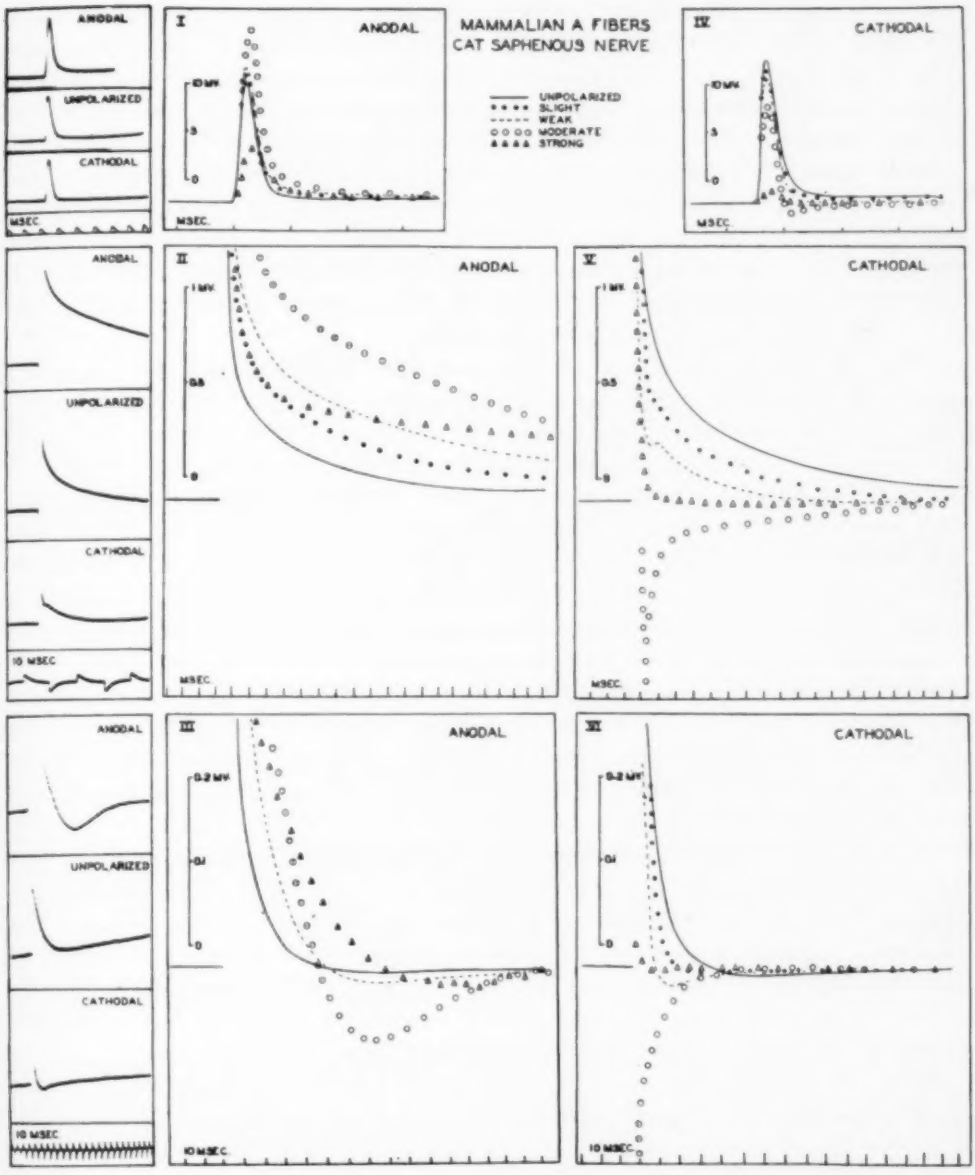

FIG. 3. Effects of polarization on mammalian A fiber action potentials. Each diagram (I–VI) consists of superimposed tracings of records taken at the indicated amplification and speed with various strengths of polarization. The effects at each pole are shown by 3 diagrams at 3 different amplifications and speeds. Actual records at zero, moderate anodal and weak cathodal polarization reproduced at left of each pair of diagrams. Slow tracings corrected for drift of base line seen in records. Electrode arrangement 3; interpolar distance, 24 mm.; interlead distance, 17.5 mm.; conduction distance, 10 mm. 5/11/39.

amphibian fibers, but as the polarization is strengthened, the development of more positive after-potential may curtail the negative after-potential. Cath-

odal polarization of moderate strength may completely suppress the negative after-potential of either amphibian or mammalian A fiber responses (Fig. 2, V; Fig. 3, V); but with some frog sciatic nerves a long low negative after-potential persists in spite of cathodal polarization strong enough to reduce the spike to a small fraction of its normal height.

The protracted negative after-potential recorded from veratrinized nerve increases at the anode and decreases at the cathode as the usual negative after-potential does (Fig. 4). The latter ordinarily manifests at the anode prolongation of its rising-phase too slight to record under the usual conditions, but the anodal prolongation of the rising-phase of the veratine after-potential is great enough to be conspicuous.

The effects of polarization on the positive after-potential are somewhat more difficult to determine because this potential is (in A fibers) relatively small, and because it varies in magnitude and duration with the condition of the fibers. It consists of two elements, P_1 and P_2, one or both of which may be absent in a single normal response (15, pp. 213–215, and references there). The presence of these two elements, overlapping in time, is revealed by a definite change in the slope of the positive potential curve under certain conditions, but the two elements can not always be so distinguished, and no such landmarks are found in the records of the polarization experiments. P_1 and P_2 can however be differentiated to some extent by their chronological position in the response. The experimental observations indicate, as shown by the following summary of them, that both P_1 and P_2 are increased at the anode and decreased at the cathode. The increase at the anode has already been referred to as interfering at times with prolongation of the negative after-potential there.

Amphibian A fibers normally exhibit relatively little after-positivity, and they do not manifest a detectable amount of P_1 under anodal polarization, perhaps because the increase of negative after-potential obscures it. The anodally increased negative after-potential converts the recorded potential from positive to negative during the interval marked by increasing positivity in the unpolarized response (Fig. 2, III), but marked P_2 develops at longer intervals, and the maximum positivity falls later than in the unpolarized response. The change of potential sign from positive to negative does not occur in mammalian A fiber responses, presumably because the development of P_1 is able to counterbalance the increased negative after-potential; it is only with polarization strong enough to result in decreased spike height at the anode that the change of sign occurs and the maximum of the positive after-potential is postponed (Fig. 3, III). The positive after-potential decreases as the negative after-potential does under anodal polarization strong enough to result in decreased spike height (Fig. 3, I, II, III, strong polarization), but quantitatively significant positive after-potential records at such strengths of polarization are hard to obtain at the anode because of a tendency to shifts of potential and "spontaneous" activity (Fig. 2, slow record at moderate anodal polarization; Fig. 3, III, end of tracing at strong

polarization). These disturbances frequently appear under moderate polarization and become greater with increasing strength of polarization; they are particularly marked after stimulation.

The characteristic decrease of the positive after-potential at the cathode is clearly seen in amphibian A fiber responses (Fig. 2, VI), because in these responses all the positive after-potential is subsequent to negative after-potential, but the decrease is obscured in mammalian A fiber responses by

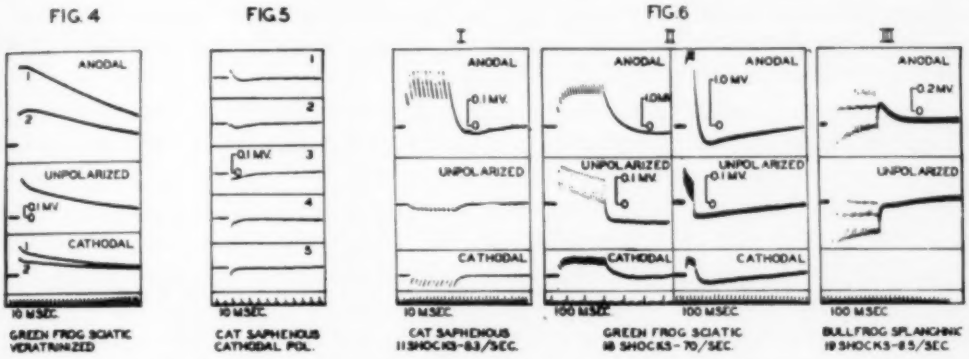

FIG. 4. Negative after-potential of veratrinized nerve polarized. Records 1, moderate polarization; records 2, stronger. All records at same amplification. Electrode arrangement 1; interpolar distance, 83 mm., interlead distance, 6 mm.; conduction distance, 9 mm. 3/21/38.

FIG. 5. Modification of early after-positivity under cathodal polarization. Record 1, unpolarized; records 2–5, weak, moderate, strong and stronger polarization. All records at same amplification. Electrode arrangement 1; interpolar distance, 51 mm.; interlead distance, 6 mm.; conduction distance, 21.5 mm. 1/30/38.

FIG. 6. Repetitive responses in nerves under moderate polarization. I. All records at same amplification. Electrode arrangement 1; interpolar distance, 51 mm.; interlead distance, 6 mm.; conduction distance 21.5 mm. 1/30/38. II. Cathodal records at same amplification as unpolarized. Electrode arrangement 1; interpolar distance, 39 mm.; interlead distance, 6 mm.; conduction distance, 22 mm. 11/14/38. III. Both records at same amplification. Electrode arrangement 3; interpolar distance, 7.5 mm.; interlead distance 3 mm.; conduction distance, 6 mm. 2/3/39a.

the exposure of P_1 (and diphasicity; Fig. 3 and 5). This exposure is the result of removal of negative after-potential which to some degree conceals the early positivity in the response of the unpolarized nerve (Fig. 3, VI, slight, weak and moderate polarization; Fig. 5, weak and moderate polarization). The positivity thus revealed is of shorter duration than the normal positivity (Fig. 5, records 1 and 2), and becomes progressively shorter as the strength of polarization is increased (Fig. 5, records 3–5). In mammalian as in amphibian A fiber responses, therefore, cathodal polarization decreases the positive after-potential.*

* Confusion arising from the negative after-potential changes accounts for the misstatements regarding the behavior of the early positive after-potential under polarization in the first account of these experiments (*Amer. J. Physiol.*, 1938, *123:* 79)

The after-potential changes under polarization produce conspicuous differences in the cumulative potentials recorded from series of responses in unpolarized and polarized nerve (Fig. 6, I and II), but repetition brings out no important new features. The negative after-potential, already large in the single response at the anode, is increased somewhat by summation with repetition. Comparison of the slow cathodal record in Fig. 6, II, with Fig. 2, VI (moderate polarization in both cases) illustrates the cumulation of positive after-potential, largely P_1 under these conditions, with repetition of amphibian A fiber responses (12, p. 41). The increase of positive after-potential under anodal polarization in Fig. 6, II, is rather less than is usually seen with these fibers stimulated repetitively.

Recovery of exitability has been followed in a few polarized nerves by the method of recording the height of response to a fixed submaximal stimulus at various intervals after a conditioning response and comparing it with the height of unconditioned response to stimuli of various strengths. The polarizing electrode was applied at or near the point of stimulation in such experiments, instead of at the recording lead. The changes in recovery observed—more rapid recovery and greater tendency to supernormality with anodal polarization, slower recovery and suppression of supernormality with cathodal polarization—are in keeping with the effects of polarization on the negative after-potential.

C fibers. As will be shown, C fiber responses are affected by polarization essentially as A responses are, but the much greater prominence of the after-potential changes in C responses makes the spike changes relatively inconspicuous. The interpretation of such potential changes as are clearly not referable to after-potential is interfered with by the temporal dispersion occurring in such slow responses even with short conduction distances, and by the great tendency of C responses to be diphasic. The spike changes are so obcured by these factors as to defy quantitative estimation and to offer no firm basis for gauging the degree of polarization; the polarization of C fibers is therefore designated "weak," "moderate," or "strong" etc. somewhat arbitrarily, though not without regard to the intensity of the effects produced and to the effects of comparable voltages and amperages in A fibers.

C fibers are less satisfactory than A fibers in polarization studies for the further reason that their responses do not readily and completely revert to the original unpolarized form after even very mild polarization. (Brodie and Halliburton, p. 185, called attention to the permanent effects of a constant current in non-medullated nerves, in contrast to medullated.) For instance, the height of the unpolarized spike varies, usually growing lower, as an after-result of either anodal or cathodal polarization; and the positive phase of the unpolarized response is frequently much smaller for an indefinite period after cathodal polarization has been applied and removed than it was originally, while it tends to be larger subsequent to anodal polarization. Nerves showing marked after-effects have been avoided as far as possible in the illustrative material used in this paper.

The presence of A fibers in nerves used for experiments on C fibers results in a shift of the start of the C fiber potential record toward the negative side with anodal polarization because of the increased negative after-potential of the A fibers (Fig. 8, I and II); when this occurs, an approximate correction may be made for it. In spite of all the difficulties, there is no doubt that the component potentials of C responses change qualitatively under polarization as do those of A responses, but quantitative estimates of the amount of change are unreliable.

Superimposed tracings of records in which the polarization effects on the spike are to a certain extent separable from those on the negative after-

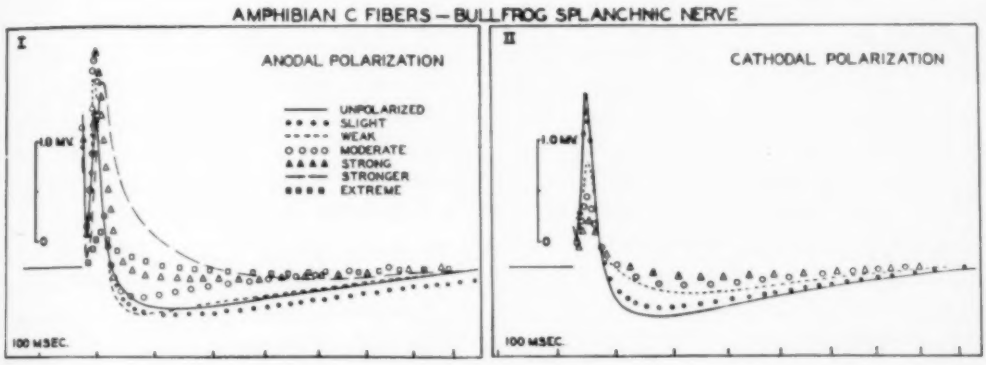

FIG. 7. Effects of polarization on C fiber action potentials. Superimposed tracings of records. Electrode arrangement 2; interpolar distance, 4 mm.; interlead distance, 8 mm.; conduction distance, 6 mm. It was shown by increasing the strength of stimulation that excitability changes at the remote pole did not account for the observed changes in spike height. 4/8/39a.

potential are presented in Fig. 7, for comparison with the results in Fig. 8 in which negative after-potential obscures the behavior of the other components of the response.

In the bullfrog splanchnic nerve responses represented in Fig. 7, the height of the spike increased at the anode to a maximum (not reproduced; it occurred with a strength of polarization intermediate between strong and stronger) about 60 per cent above the unpolarized height, and fell off with stronger polarization. The amplitude of the characteristically large positive after-potential (2; 14, p. 301) increased at the anode with slight polarization, and in its early portion with weak polarization. With moderate and strong polarization, however, it was reduced in amplitude and duration at the anode; with stronger polarization it was replaced by negativity lasting more than 200 msec. With extreme anodal polarization, the whole response largely disappeared.

In the series of bullfrog splanchnic nerve responses represented in Fig. 8, II, on the other hand, there was no increased after-positivity at the anode with any strength of polarization; the whole curve shifted towards the nega-

tive side from the very start of the response even with weak polarization. With stronger and extreme polarization, the potential was recorded as one prolonged negative wave (cf. the tracings for these two strengths of polarization in Fig. 7, I). The maximum negativity reached in the experiment of Fig. 8, II, was about twice the unpolarized spike height. The fusion between the spike potential and the negative after-potential at the anode was less complete in other experiments on bullfrog C fibers than in the ones used for

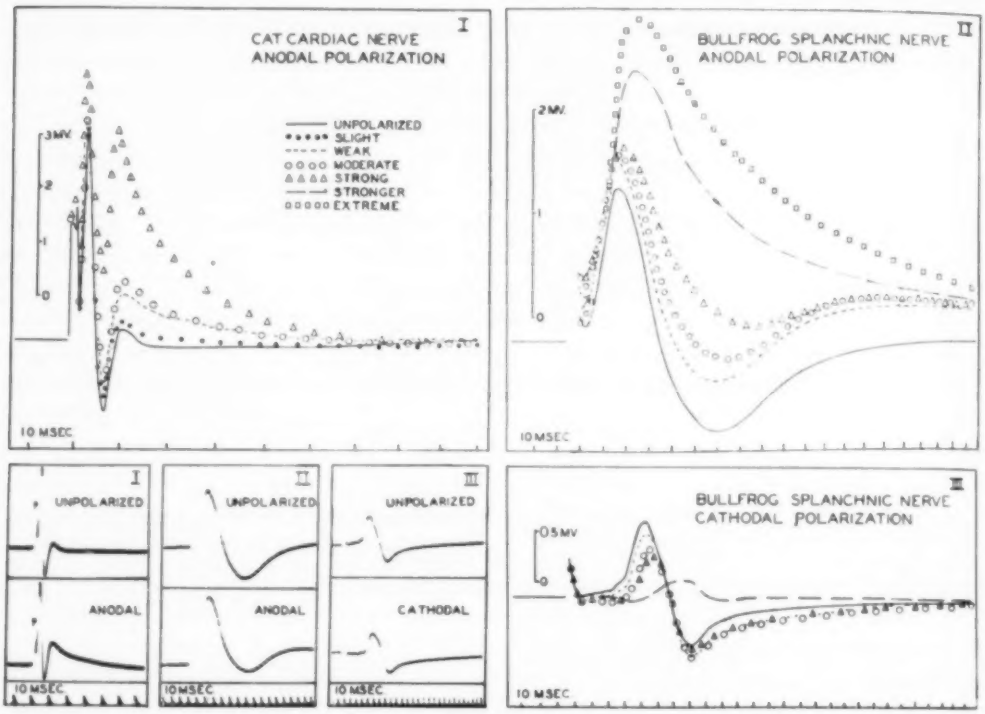

FIG. 8. Effects of polarization on C fiber action potentials. Superimposed tracings of records. Records at zero and moderate polarization reproduced. I. Electrode arrangement 3; interpolar distance, 16.5 mm.; interlead distance, 12 mm.; conduction distance, 2.5 mm. 5/11/39c. II. Electrode arrangement 3; interpolar distance, 19.5 mm.; interlead distance, 19.5 mm.; conduction distance, 4.5 mm. 4/8/39a. III. Electrode arrangement 2; interpolar distance, 11 mm.; interlead distance, 7 mm.; conduction distance, 15 mm. 3/24/39a.

the figures, and tended to be still less complete in cat C fibers; the cat C fiber responses illustrated by Fig. 8, I, show an unusually distinct separation between the spike and the negative after-potential because of diphasicity.

Are both the spike and the negative after-potential of C fiber responses, like those of A fiber responses, increased and prolonged at the anode? Convincing evidence for the increase of the constituent fiber spikes is not available from such experiments on multifibered nerves as those of Fig. 8, I and II, if it be assumed that negative after-potential from the faster C fibers develops promptly enough to raise the crest of the composite spike. Even

if this assumption be granted however, such spike increases as are accompanied by increased positive after-potential (Fig. 7, I, slight and weak polarization), must be due to actual increases in the constituent spikes. In the experiment of Fig. 7, the spike height increased to 50 per cent above its unpolarized height with polarization too weak to produce any confusing negative after-potential; this is of the order of the usual maximum increase observed at the anode with composite spikes of A fibers. Similar records of simultaneous increases of spike height and positive after-potential were obtained with cat C fibers, and it is concluded that the spikes of both amphibian and mammalian C fiber responses are increased in amplitude at the anode.

No data were obtained on the duration of C fiber spikes under polarization; is it possible that lengthened spike duration, plus the effects of temporal dispersion, accounts for all or a considerable part of the prolongation of negativity recorded? Erlanger and Blair's illustrations of single A fiber spikes show not more than doubled length at the anode before block; unless C fiber spikes differ very greatly from A in this respect, lengthened duration of the constituent single spikes can not account for the lengthening of negativity observed at the anode with C fiber nerves. The temporal dispersion of the conducted spike will increase at the anode if C fiber responses are slowed there as A fiber responses are (3, p. 639). It is clear from the records (*e.g.* Fig. 8, II) however that the slowing of conduction is not marked, and calculation based on the known range of conduction rates of bullfrog C fibers (0.6 to 0.2 m. per sec. (14, p. 299)) shows that even halving the rate will not extend spike negativity as much as 100 msec. with the conduction distances used in these experiments. Development of negative after-potential at the anode, therefore, suggested by inspection of the records as well as by the behavior of A fiber responses, probably accounts for most of the prolonged negativity observed at the anode in C fiber responses.

An example of the anodal increase of negative after-potential following repetitive stimulation of C fibers is included in Fig. 6, III; except for the reduced frequency of stimulation and the greater positive after-potential (note the positive level of potential maintained during stimulation of the unpolarized nerve) the records are similar to those of repetitive responses from A fibers (Fig. 6, I and II).

At the cathode the C fiber spike seems to decrease in height about as that of A fibers does; with the strength of polarization giving at the anode the greatest spike height to which increased negative after-potential certainly does not contribute, the height at the cathode is decreased to about 60 per cent normal (Fig. 7); this may be compared with the decrease of spike at the cathode to 40–80 per cent of normal in A fibers with polarization giving maximal spike increases at the anode. Frank negative after-potential is absent from single responses of normal bullfrog C fibers, and, as expected from the results with A fibers, is not developed at the cathode; parallel decreases of spike and positive after-potential throughout the applicable range of polarization strengths are therefore ordinarily observed with bullfrog C

fibers (Fig. 7, II), and with cat C fibers as well. Occasionally however, particularly in nerves already subjected to numerous periods of polarization, increases of after-positivity are observed with cathodal polarization (Fig. 8, III) as they are with cathodal polarization of mammalian A fiber responses (Fig. 3, VI; Fig. 5). It is believed that in the case of such C fibers, some negative after-potential overwhelmed by positive after-potential, lies concealed in the unpolarized response; the explanation of the apparent increase of positivity at the cathode is then the same as the explanation of the same phenomenon in mammalian A fibers (p. 144).

The positive after-potential in C fiber responses which has been described as increasing with the spike at the anode (until its behavior is obscured by the increase of negative after-potential) and decreasing with the spike at the cathode, is P_1; study of P_2 in polarized C fiber responses was abandoned because of difficulty in maintaining a sufficiently steady base line for the length of time required for an observation.

Discussion

Observations similar in principle to those incorporated in this report were published by Verzár in a series of papers from 1912–1928; he studied chiefly the potentials developed by frog sciatic nerves stimulated tetanically, recorded diphasically by a string galvanometer. Because cathodal polarization reversed rather than merely decreased the prolonged potentials manifested at the anode, he came to the conclusion (28, p. 16) that his observations were not explicable as prolongation of the action potential at the anode with distortion by instrumental inertia. He applied the term "depolarization wave" to the level of potential maintained during stimulation and its gradual return to the resting level following stimulation (26), because the potential was opposite in direction to the extrapolar electrotonic current. The "depolarization wave" was followed by a potential wave in the reverse direction, the positive after-potential, which Verzár correctly concluded was related to the "depolarization wave" (27). However, though he used Hering's designation of positive after-potential, he concluded that this wave was actually increased polarizability rather than positivity (27, p. 253), since both anelectrotonus and catelectrotonus were increased during its passage, and positivity would have decreased catelectrotonus.

Verzár's conception of the first prolonged potential as depolarization was adopted by Samojloff and Kisseleff, who believed they recorded it separately from the action potential on the anodal side of a polarized region when an impulse approaching the region from beyond the cathode was "blocked" at the cathode (22, p. 482; on the anodal side, "von den Aktionsströmen sieht man so gut wie gar nichts mehr"). They were unable to explain why it was not recorded when the impulse approached the polarized region from beyond the anode, although as they were then recording on the cathodal side and as usual monophasically, the explanation now seems obvious. When the "depolarization wave" appeared in records from the anodal side, the impulse actually could not have been completely blocked at the cathode.

Ignorance of the existence of the negative after-potential in unpolarized nerve, combined with (i) neglect of Hermann's warning statement (20, p. 270, footnote) that any slow potential from unpolarized nerve would balance out in diphasic recording and (ii) unintentional recording of anodally increased negative after-potential disguised as positivity at the cathode, resulted in the misinterpretations of experimental observations just cited, as in the general adoption (*e.g.* Ebbecke) of the changed polarizability view of the interaction between polarization and activity. Apparently, in the sixty years since Hermann's admission that the polar changes of the action potential known at that time were insufficient to account for the changes of polarizing and polarization currents with activity, Boruttau (4, p. 165) has been the only worker to show any hesitation at accepting the hypothetical changed polarizability. His hesitation seems to have been due to his impression that the action potential had components long enough to account for the prolonged changes of potential observed in polarized nerve (cf. 25, pp. 301–302). He expressed the opinion (5, p. 300) that the increment-decrement law of Hermann explained all the observed phenomena adequately, and while this is not true if the application of the law is restricted to the spike potential, the law is the least hypothetical and most convenient formulation of the facts when the negative after-potential is included in it. Recent indications (8 and references there; 9) of the possible number and variety of potential sources and polarizable structures in the nerve "membrane," emphasize the desirability of being as specific as may be in regard to any observed potential change. The observations reported in this paper are so readily described by the increment-decrement law that it is superfluous to do so here (see summary below, 4).

These experiments tend to confirm certain more or less well-established conclusions regarding the negative after-potential (15, pp. 214–216 and references there). (i) The negative after-potential begins early enough in the response to affect the height of the composite spike; any other interpretation of such potential forms as are illustrated in Fig. 8, II is almost impossible. (ii) The negative after-potential has a distinct rising-phase that under certain circumstances outlasts the main part of the spike (Fig. 4). (iii) Increased negative after-potential however produced is generally accompanied by shortened relative refractoriness and increased supernormality, while decreased negative after-potential is accompanied by changes of recovery of excitability in the opposite direction; polarization is evidently one of the after-potential determinants which does not disturb this relationship of after-potential to recovery (p. 144). (iv) The late positive after-potential, P_2, is a concomitant of the negative after-potential and varies in size with it in polarized nerve as in nerve subjected to other influences (Fig. 2, III, VI; Fig. 3, III, VI); this is in line with Gasser's suggestion of an intimate connection between these two potentials (13, pp. 167–169). Although not concerned with the negative after-potential, the variation in the size of the early positive after-potential, P_1, with variation in size of the spike, particularly

its increase in anodally polarized C fibers (Fig. 7, I), may be mentioned in support of the other part of Gasser's suggestion regarding the interrelationship of the component parts of the nerve action potential, *i.e.* the intimate connection between the spike and P_1.

Whatever the structures or mechanisms producing the spike potential (with its companion, P_1?) and the negative after-potential (with its companion, P_2?), the negative potentials are both reversible discharges of polarizable systems, and imposed polarization brings out certain similarities and certain dissimilarities between the two systems. They are alike (i) in being polarized in the same direction, (ii) in being approximately equally sensitive to polarization, (iii) in having their discharge time (rising-phase) prolonged when their charge is increased under anodal polarization, and (iv) in not being polarizable in the opposite direction by any strength of applied (cathodal) polarization, *i.e.* neither is ever manifest as positive potential initially. They are unlike (i) as already known, in their time relations, and (ii) in their polarizability; *i.e.*, in the degree to which their charges are increased under anodal polarization. At the anode, the spike mechanism does not at best acquire much more than twice its normal charge before it ceases to function, but the negative after-potential mechanism takes on a charge many times normal. When the after-potential mechanism fails to discharge with stimulation under polarization, it is apparently because the spike mechanism has failed, the discharge of this mechanism (the spike) being a necessary prerequisite for the discharge of the other (the negative after-potential).

Summary

1. At the anode of a constant current applied to a green frog sciatic or cat saphenous nerve (A fibers), the following changes in the amplitude of the components of the action potential are observed:

(a) The spike increases in height with increasing strength of polarization up to a maximum usually 20–40 per cent above the polarized height; if the polarization is increased further, the spike height decreases. Successive block of fibers in the nerve with increasing strength of polarization probably accounts in large part for the differences between these observations and those on single fibers.

(b) The negative after-potential increases in size up to several times its unpolarized size, and increases in duration unless it is shortened by (c).

(c) The late positive after-potential increases in size and duration in keeping with the increase in size of the negative after-potential.

2. At the cathode of a constant current applied to A fibers:

(a) The spike decreases (to 40–80 per cent of normal height with the strength of polarization giving the maximum spike at the anode); the decrease varies with the strength of polarization.

(b) The negative after-potential is decreased and may completely disappear.

(c) Early positive after-potential may appear to be increased because of (b), but is not; late positive after-potential is decreased.

3. The changes in amplitude of the various components of the action potential of C fibers (bullfrog splanchnic, cat hypogastric, vagus or other autonomic nerve) are in general the same as those just described for A fibers, but the after-potential changes are relatively greater. Under favorable circumstances, increase of early after-positivity with anodal and decrease with cathodal polarization may be observed.

4. These observations may be simply and satisfactorily described by the statements: (i) the negative after-potential, like the spike, is increased in amplitude at the anode and decreased at the cathode; and (ii) the positive after-potentials are affected by polarization as are the negative potentials which they succeed.

5. These observations are consistent with already known properties of the components of the action potential.

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STIMULATION OF PERIPHERAL NERVE TERMINATIONS BY ACTIVE MUSCLE

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A SINGLE shock applied to the distal segment of a severed ventral root results in the appearance of a group of impulses which reflect from the periphery into a fraction of the stimulated ventral root fibers, into unstimulated but neighboring ventral root fibers, and into fibers of the corresponding dorsal roots (9). Centripetal discharges have been observed in ventral roots or motor nerves of eserinized preparations (10, 4, 6, 5) These centripetal discharges have been regarded as arising at the motor nerve endings (under the influence of eserine) and coursing both orthodromically into the muscle there to cause the repetitive responses characteristic of eserinized muscle (3) and antidromically along the motor nerve (ventral root) where they may be recorded. Two lines of evidence stand contrary to this view. Eccles, Katz and Kuffler, (5) point out that the association of "repetitive" motor nerve responses with repetitive muscle response is not invariable. Secondly the centripetal responses have been observed in non-eserinized preparations, and in root fibers other than those occupied by the efferent volley to the muscles.

Afferent activity in dorsal roots would naturally ensue upon active contraction of the muscles (12), but the dorsal root activity at present under consideration does not possess the features to be expected in the case of discharges from any known tension receptors.

It is the purpose of the present study to show the origin and nature of the secondary centripetal discharges in the normal (*i.e.* non-eserinized) preparation. Some of the pertinent observations have been presented briefly in a preliminary note (9).

The animals used in these experiments were cats, and two rabbits, one of which showed characteristic secondary centripetal activity. According to Feng and Li (6) normal "after discharge" occurs also in rats. The animals were narcotized with Dial (0.5 ml. per kg.). Selected dorsal and ventral roots were exposed by laminectomy and severed from their connection with the spinal cord for the purpose of placing stimulating and recording electrodes.

When the seventh lumbar ventral root (L.7 V.R.) is stimulated by a single maximal shock, recording leads on that root reveal the spike potential of the stimulated volley (the primary motor volley) followed, after some 2.5 msec., by an asynchronous discharge lasting, on the average, ca. 5 msec. (Fig. 1A). An essentially similar result is obtained if the first sacral ventral root (S.1 V.R.) is substituted (Fig. 1C). The records of Fig. 1 were all obtained with one of the recording leads at the killed end of the root in question. When both recording leads are placed on the intact root distal to the

stimulating electrodes the phases of the potentials of the primary motor volley and of the secondary discharges are reversed indicating clearly that the primary motor volley passes distally to the muscles, whereas the secondary impulses are centripetal (cf. Lloyd, 9, Fig. 1F, L, M).

The discharge of 1 B of Fig. 1 is recorded from the S.1 V.R., and results from a single shock delivered to the L.7 V.R. The primary motor volley is of course not recorded. The secondary centripetal impulses are present, although the discharge is not as intense as it is in the L.7 V.R. The latency of

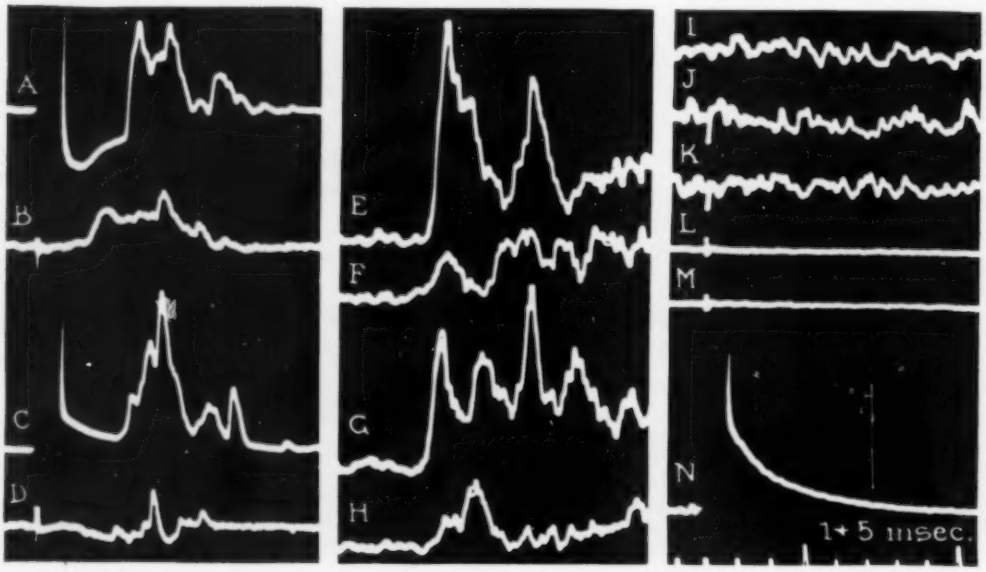

FIG. 1. Distribution of secondary centripetal discharges in ventral and dorsal roots following stimulation of ventral roots. Time in 1 and 5 msec. divisions below record N. In all figures where there are two time designations, these are for the small and large divisions respectively. Further description in text.

the secondary centripetal discharge is shorter in 1B than in 1A or 1C, being ca, 1.6 msec., as compared with 2.5 msec. The differential is not always so great, although it is generally present unless other factors are operative. Figure 1D shows the secondary centripetal discharge in the L.7 V.R. resulting from a S.1 V.R. shock.

From a consideration of Fig. 1 (A to D) it becomes apparent that the occurrence of secondary centripetal discharges in ventral roots does not depend upon the identity of the nerve structures transmitting the primary motor volley and the secondary centripetal discharges. Secondary centripetal discharges, on the other hand, are not encountered if several segments intervene between the "donor" and "recipient" ventral roots. The spatial limits to the distribution of the secondary centripetal discharges appear, therefore, to be set by the extent of spatial fusion in the distribution of the several ventral roots to the muscles. Spatial fusion is extensive in the

limb musculature (16), which no doubt accounts for the substantial secondary centripetal discharges encountered in ventral roots adjacent to a root occupied by the primary motor volley. Inasmuch as the secondary centripetal discharges do occur in roots not occupied by the primary motor volley, they are not repetitive discharges with respect to that volley.

Secondary centripetal impulses occur in corresponding dorsal roots with comparable latency. Figure 1(E, F) shows these impulses recorded from the seventh lumbar dorsal root (L.7 D.R.) and the first sacral dorsal root (S.1 D.R.) following single shock stimulation of the L.7 V.R. Similarly Fig. 1(G), H shows secondary centripetal responses in the S.1 D.R. and L.7 D.R. to stimulation of the S.1 V.R. The earliest dorsal root secondary centripetal responses occur usually after 1.6–1.7 msec. latency. Again, as in the case of the ventral root secondary centripetal responses, it will be noted that the dorsal root secondary centripetal responses are greatest when the ventral root of the same segment is stimulated.

Figure 1 (J to N) illustrates the ineffectiveness of centrifugal volleys in dorsal roots with respect to the initiation of secondary centripetal discharges. In 1J, the L.7 D.R. is stimulated while recording from the S.1 D.R. In 1K, stimulating and recording leads are reversed. The activity recorded in J and K is similar to that recorded in I without stimulation, and represents the normal and constant flow of afferent activity over the dorsal roots (1). For records L and M recording leads were placed on the L.7 V.R. and S.1 V.R. respectively, in each case the dorsal root of the same segment being stimulated. Again no secondary centripetal activity results. Finally in N, both stimulating and recording electrodes were placed on the S.1 D.R. A primary spike potential is recorded unaccompanied, however, by any sign of secondary centripetal activity.

It is clear from the observations of Fig. 1 that secondary centripetal discharges result only when a ventral root is stimulated and occur in fibers, both dorsal and ventral root, having a degree of anatomical fusion of peripheral distribution.

It has been noted that the latency for secondary centripetal discharge in a stimulated ventral root is longer than that in unstimulated ventral roots or in dorsal roots. Differences other than latency exist between the secondary centripetal discharges of stimulated and unstimulated roots. These are: (i) the relative size of those discharges, and (ii) the size of the primary motor volley necessary to procure threshold secondary centripetal discharges in dorsal and stimulated ventral root fibers.

Figure 2 presents a graph relating the amplitudes of the dorsal root and stimulated ventral root secondary centripetal discharges, plotted in similar arbitrary units but on different scales, to the size of the primary ventral root volley expressed in per cent of maximum. The dorsal root secondary centripetal discharge is much greater than that of the ventral root. This fact may be appreciated also in Fig. 1, 3 and 4 by noting that the ventral root responses have been recorded regularly at an amplification five times that

used for the dorsal root responses. Figure 3 further indicates the much lower threshold for production of the dorsal root secondary centripetal responses than for the production of the ventral root secondary centripetal responses.

Thus there are differences in latency, size and threshold to distinguish between the secondary centripetal discharges in dorsal and stimulated ventral roots. These differences are consentient in indicating that the motor fibers, as a result of the passage of the primary motor volley, are partially refractory to the excitatory process causing the secondary centripetal discharges.

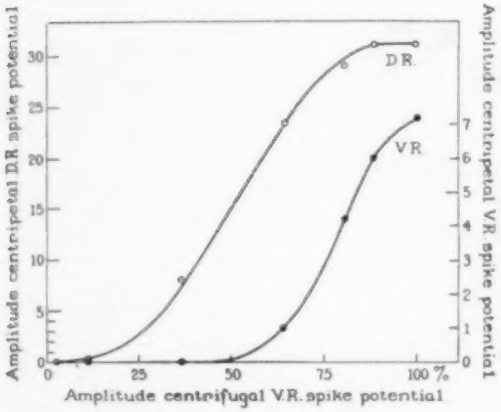

FIG. 2. Graph relating the size and threshold of secondary centripetal discharges in a dorsal root and a stimulated ventral root. The first spike potential of the discharges was selected for measurement and illustration merely because some part of the complex discharge must be chosen. Other parts of the secondary centripetal discharges respond similarly to ventral root shocks of increasing intensity.

Elements of the secondary centripetal discharge. It has been shown that the secondary centripetal discharge is asynchronous, having a duration of ca. 5 msec. This could be due to repetitiveness, or to a variety of conduction times occasioned by circuits of various lengths. To decide between these two alternatives, advantage is taken of the fact that the constituents of the lumbosacral cord break up into three major groups; the gluteal and other nerves innervating muscles about the hip, the hamstring group to muscles of the thigh, and the sciatic group to muscles of the leg. These groups are characterized by different average conduction distances between the spinal roots and the muscles concerned; each group may be interrupted in isolation from the others. The results of experiments in which selective denervation of these muscle groups was practised are illustrated in Fig. 3.

Figure 3 (A, E, I) shows the ventral root (L.7) secondary centripetal discharges in three experiments preceding surgical intervention in the leg. Similarly Fig. 3 (C, G, K) shows the dorsal root (also L.7) secondary centripetal discharges in the same three experiments and under the same conditions. Denervation of hip muscles changed the ventral root secondary centripetal discharge from that recorded in 3A to that recorded in 3B, and the dorsal root secondary centripetal discharge from that in 3C to that in 3D. The initial segment of the secondary centripetal discharge is depleted in each case;* the terminal segment is retained.

* The spike potential indicated by an arrow in Fig. 3D serves to illustrate one of the sources of confusion inherent in denervation experiments. This spike potential is not present in the normal record 3C. It has appeared *de novo* as a result of nerve section. Its latency is ca. 0.5 msec. shorter than that of the first activity in 3C. It may be identified

When the hamstring nerves are severed the secondary centripetal discharge in ventral roots is changed from that recorded in 3E to that recorded in 3F, while the dorsal root secondary centripetal discharge is changed from that in 3G to that in 3H. Figure 3 (E to H) shows that the intermediate segment of the secondary centripetal discharge is obliterated by denervation of the hamstring group. In similar fashion the observations 3I to 3L

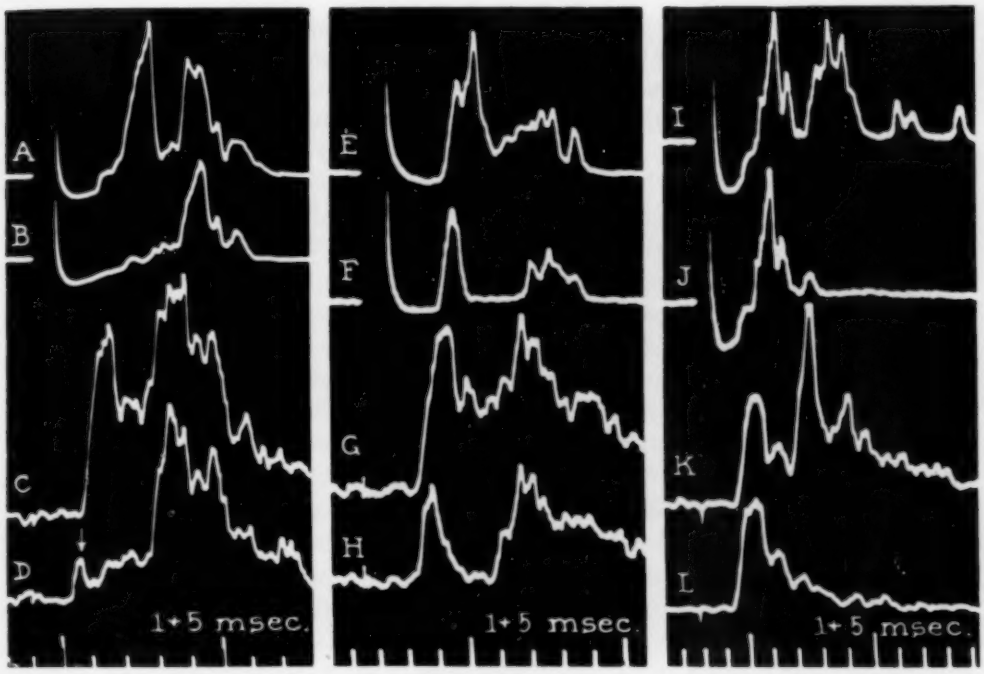

FIG. 3. Removal of specific segments of dorsal and ventral root secondary centripetal discharges by selective denervation of muscle groups. The spike potential introduced in record D after nerve section is a "denervation artefact."

show that the terminal portion of the secondary centripetal discharge is removed by section of the sciatic nerve (compare J with I and L with K), whereas the initial portion is retained almost perfectly intact.

There can be little doubt that the significant factor for dispersion of the secondary centripetal discharge is variety of conduction distance to and from the several groups of nerve endings, efferent and afferent, and that the latency of each segment of the discharge is related to the total conduction distance involved. It will be noted that the major discharges of any particular group remaining intact occur with a shorter latency in the dorsal roots than in the stimulated ventral roots. The initiation of the later segments of

as due to stimulation of D.R. fibers at the cut end of the common nerve trunk by closely intermingled active V.R. fibers (cf. 7). Another source of error arises in the stimulation of cut nerve ends by active muscle (11). Careful preparation usually reduces these "artefacts" to insignificance.

the secondary centripetal discharge in the stimulated ventral root therefore appears to be subject to the same conditions of partial refractoriness that hold for the initiation of the shortest latency secondary centripetal discharges (cf. Fig. 1 and 2). The latency differential between dorsal and stimulated ventral root secondary centripetal discharges would necessarily hold for each segment of those discharges if the secondary centripetal discharges are initiated in the terminal regions of "recipient" fibers at a relatively fixed time with respect to the arrival of the primary motor volley at the various motor fiber terminations, conduction distance being the major variable.

In occasional experiments one or two centripetal discharges occur in a few fibers after the completion of the major secondary centripetal discharge (cf. for example Fig. 3I). It is possible that these atypical discharges are repetitive in nature.

Factors contributing to the latency of secondary centripetal discharges. Nerve conduction time is a significant factor in latency of secondary centripetal discharges, but nerve conduction alone cannot account in full for the latency of even the earliest of these discharges for, as seen in column 7 of Table 1, the latency of secondary centripetal discharges is 0.54 to 0.8 msec. longer than that of "Hering phenomena" (stimulation of nerve by nerve) conducted over nerve paths of comparable length. The discrepancy between the latency of secondary centripetal discharge and the Hering phenomenon is also seen in Fig. 3(C, D). The exclusion of nerve stimulation by nerve as an explanation of the secondary centripetal discharges does not rest upon the evidence of differential latency alone. The fact that secondary centripetal discharges reflect into a *maximally* stimulated "donor" ventral root (Fig. 1, 3 and 4), whereas Hering phenomena do not is a powerful argument against

Table 1

Nerve-muscle group	Time from stimulus to muscle action potential	Conduction time, muscle to root	Sum of 2 and 3	Latency of secondary centripetal discharge	Latency of Hering phenomenon on sciatic N. at equivalent conduction distances	Excess over over 6
1	2	3	4	5	6	7
Hip*	1.1	0.5	1.6	1.64	1.1 (10 cm.)	0.54–0.56
	1.15	0.45	1.6	1.66†		
Hamstring	1.5	0.76	2.26	2.3	1.76 (16 cm.)	0.54
Sciatic	2.0	1.4	3.4	3.5‡	2.7 (24 cm.)	0.8

* This experiment is illustrated in the preliminary paper (Lloyd, 9, Fig. 1B, C, D, E).

† This value was obtained at the beginning of the experiment. When the active muscle field was restricted by nerve section a denervation artefact appeared with shorter latency, obscuring the normal onset of the secondary centripetal discharge.

‡ The records from which this figure was obtained contain a persistent denervation artefact emanating from the hip region. This artefact, however, did not encroach upon the secondary centripetal discharge.

an analogy between the two phenomena. Accordingly the stimulating agent causing the secondary centripetal discharges and that causing the Hering phenomenon may not be homologized for the former outlasts the absolutely refractory period of the nerve fibers carrying the primary motor volley with an intensity sufficient to reexcite those same fibers during partial refractoriness. In the case of the Hering phenomenon the stimulating agent is coincident with the arrival of the donor nerve volley (13) and does not persist long enough to clear refractoriness of the donor nerve fibers. The observations presented in Table 1 indicate that it is the time dissipated in neuromuscular delay before the stimulating agent causing the secondary centripetal discharges begins to act that allows the agent to clear the refractoriness of the nerve fibers occupied by the primary motor volley.

Table 1 shows the results of experiments designed to account for the duration of the latent period of the secondary centripetal discharges and to locate the time at which these discharges are initiated at the periphery in relation to the time sequence of events following upon a ventral root shock. A comparison of the figures in columns 4 and 5 indicates that the latency of a selected segment of the secondary centripetal discharge is approximately equal to the sum of conduction time to the muscle, neuromuscular delay and conduction from the muscle to the root recording leads. The residual time (0.04–0.1 msec.) may be accounted for as utilization time. The earliest secondary centripetal discharges are initiated, therefore, shortly after the onset of the muscle action potential at the junctional region.

Although the total secondary centripetal discharge in the intact leg lasts for ca. 5 msec., the discharge from a restricted muscular field is much shorter (Fig. 3). Restriction of the donor muscular field by denervation (Fig. 3 and Lloyd, 9, Fig. 1B) or by the use of threshold responses (for example in Fig. 6) shows that the unit secondary centripetal discharge has a total duration of 1 to 1.3 msec. at the recording leads. Allowing for the duration of the individual nerve spikes in the secondary centripetal discharge, but not for conduction dispersion, it appears that the last secondary centripetal discharges of a homogeneous group are initiated in the nerve endings within ca. 0.5 to 0.8 msec. of the onset of the first of such discharges. This time coincides approximately with the crest time of the muscle action potential. The secondary centripetal discharges are initiated, therefore, by processes approximately equal in duration to and contemporaneous with the ascending phase of the muscle action potential at or near the junctional region.

Contributary evidence for the participation of neuromuscular transmission and muscle action in the sequence of events leading to secondary centripetal discharges. If muscle action is a step in the production of secondary centripetal discharges then these discharges should be susceptible to modification in a predictable manner by agents and procedures known to influence neuromuscular transmission and muscle action.

(*1*) *The action of curare.* Curare removes the secondary centripetal discharges even when administered in doses which are not quite sufficient for

the complete blocking of muscular response. Figure 4 (A, E, I) shows the secondary centripetal discharges in L.7 V.R., S.1 V.R. and L.7 D.R. in response to a L.7 V.R. shock. Figure 4 (B, F, J) recorded after the administration of curare, illustrates the removal of the secondary centripetal discharges in each case. Figure 4J is of interest for it reveals a response of recruiting nature and of much longer latency still present after a degree of curarization sufficient to remove the secondary centripetal discharges in the dorsal root and elsewhere. Positive identification is not easy, but it is most

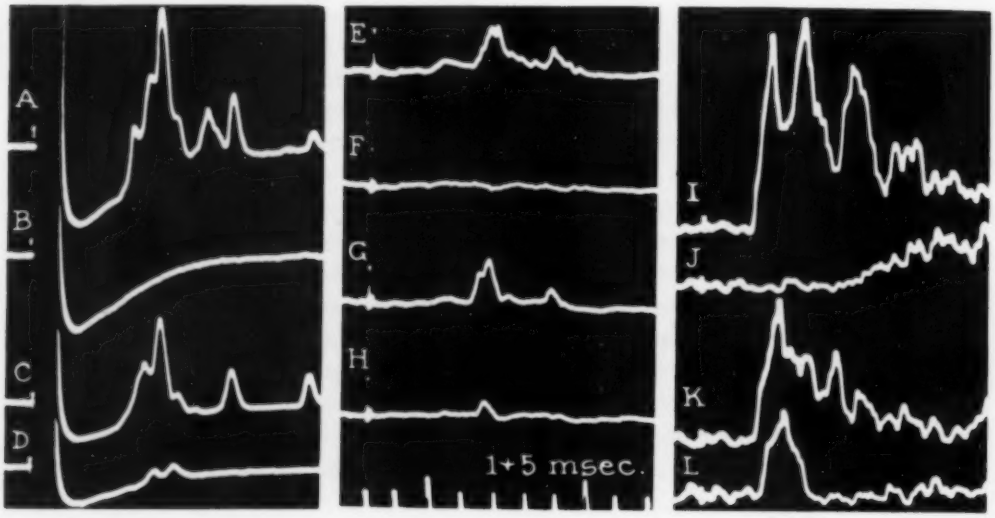

FIG. 4. Removal of secondary centripetal discharges by curare. Inhibition of secondary centripetal discharges by the Wedensky effect at the neuromuscular junction of partially curarized muscle.

likely that this response represents afferent activity initiated by the residual muscle contraction acting on tension receptors.

One might suppose that the secondary centripetal discharges should disappear only with the advent of complete curarization. Reference to Fig. 2, however, shows that the first (and lowest threshold) secondary centripetal responses appear as a result of primary motor volleys between 12 and 35 per cent of maximum size. Asynchronous recruiting afferent activity, comparable with that in Fig. 4J, appears following near threshold motor volleys. Differential threshold then is adequate to account for the segregation by curare of later afferent activity from the secondary centripetal discharge.

With small doses of curare the secondary centripetal activity is present, with latency unaltered, but with intensity diminished (compare Fig. 4C, G, K with the control observations 4A, E, I). If two shocks are delivered in succession to a ventral root of a partially curarized preparation at intervals up to 5 or more sec., the secondary centripetal activity elicited by the second of such pairs of shocks is markedly reduced. The pairs of responses

C and D, G and H, and K and L were recorded at an interval of 1 sec. in each case. The reduction in the secondary centripetal activity elicited by the second shock of each pair is readily apparent. The effect is clearly a reflection of the prolonged "Wedensky type" inhibition characteristic of partially curarized muscle (8, 2, 15).

(*2*) *The action of eserine.* It has been known for some time that the response of eserinized muscle to a single motor volley is repetitive (3). Likewise it has been in the eserinized preparation that repetitive centripetal activity has been observed (10, 6, 5). It remains to be shown, however, that the repetitive nerve activity is primarily grafted onto the secondary centripetal activity present before the administration of eserine. Figure 5 illustrates the secondary centripetal discharges evoked in one half of the L.7 V.R. by stimulation of the other half before (A) and after (B and C) the administration of eserine (0.5 mg. intravenous). Comparison of 5A and 5B reveals that the original secondary centripetal discharge group is retained and that the repetitive discharge is added thereto. Figure 5C, similar to 5B but recorded on a slower time axis, illustrates how the repetitive discharge is prolonged in time.

It is difficult in view of the other evidence presented here to escape the conclusion that the repetitive centripetal discharges recorded under the influence of eserine result from the repetitive response of the muscles to a single nerve volley, rather than *vice versa* as proposed by Masland and Wigton. The present interpretation has the additional merit of accommodating the observation of Eccles, Katz and Kuffler (5) that repetitive muscle responses caused by eserine frequently occur without discharges into the motor nerve. The explanation of the variable association would lie in the intensity of the muscle action necessary to secure centripetal discharges (cf. discussion in connection with Fig. 2 and 4).

The occurrence of centripetal discharges associated with the spontaneous fasciculation of eserinized cat's muscle, first observed by Masland and Wigton, has been confirmed.

(*3*) *The stages of neuromuscular transmission.* One of the prominent characteristics of neuromuscular transmission is the series of variations in muscle response to maintained tetanic stimulation of the motor nerve. These variations have recently been systematized by Rosenblueth and coworkers (14) into five stages of neuromuscular transmission. The influence of these stages of neuromuscular transmission on the initiation of secondary centripetal discharges has been observed, in varying degree, in all the preparations made for that purpose. The second stage is often absent, but the stages representing "treppe" and "fatigue" are regularly present. The optimal preparation for observing the treppe phases (stages 1 and 3) is that employing separate ventral roots for stimulating and recording.

Observations D to K of Fig. 5 illustrate the influence of the initial stages of neuromuscular transmission on secondary centripetal discharges and were recorded in the following manner. A single response (5D) was recorded.

The succeeding responses were recorded at intervals as a standing wave, the stimulus being synchronized with the sweep, the stimulus frequency approximating 50 per sec. There are slight variations in individual responses in addition to the slower trends; hence the double exposure effect in records E to K. In the experiment illustrated the second stage is present resulting in an initial decrease in the secondary centripetal discharge, succeeded by an increase (third stage) and "fatigue" (fourth stage). The experiment was not prolonged to the point of observing the fifth stage.

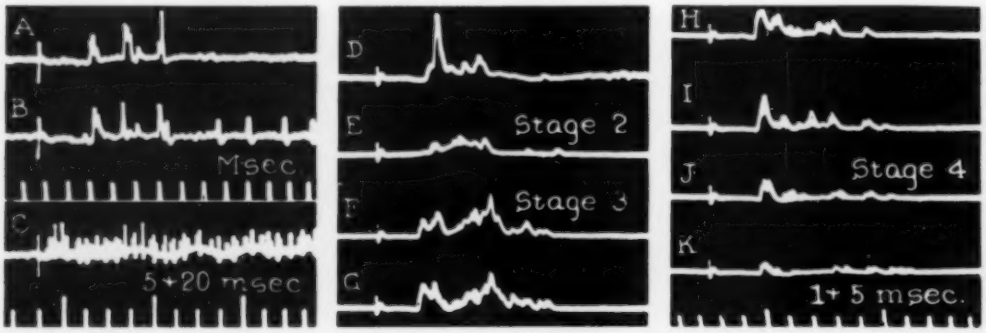

FIG. 5. Influence of eserine on secondary centripetal discharges (A, B, C). D to K—the secondary centripetal discharges reflect the stages of neuromuscular transmission. The time lines refer to the record or records immediately preceding.

In addition to the slow changes in intensity of secondary centripetal activity, Fig. 5 illustrates a fact which holds equally for secondary centripetal discharges in dorsal roots, stimulated and unstimulated ventral roots. The secondary centripetal discharges follow exactly the stimulus frequency at frequencies yielding mechanical fusion of muscle contraction. Granting that some aspect of muscle action is responsible for the initiation of secondary centripetal discharges, the last fact would tend to exclude muscle contraction as the stimulating agent. It is worth noting, since stimulation by muscle action currents is implied as the mechanism of secondary centripetal discharge in an earlier section, that the muscle action potentials remain distinct during a tetanus and furthermore that they undergo characteristic amplitude variations during the several stages of neuromuscular transmission (14).

The locus of stimulation of recipient nerve fibers. The observations on latency (Fig. 3 and Table 1) indicate that the recipient nerve fibers are stimulated at or near their terminations and not at random along some part of their course. In order to substantiate this view, control experiments to demonstrate that active muscles do not stimulate the *intact* nerve trunks (*i.e.* the preterminal portions of the nerve fibers) were fashioned as follows. In addition to the usual root preparation, a small window was made over the hamstring nerves which were located and severed. Otherwise the normal anatomical relationships within the leg were preserved. On stimulating a

ventral root, centripetal discharges comparable with those illustrated in Fig. 3F resulted. Stimulating electrodes were applied to the distal segment of the severed hamstring nerves. On stimulation of the hamstring nerves, resulting in contraction of the hamstring muscles, no secondary centripetal discharge was recorded at the spinal roots although a great length of the sciatic nerve lay immediately subjacent to the active hamstring muscles. Furthermore, stimulation of the hamstring nerves did not influence the secondary centripetal discharge resulting from stimulation at the spinal root.

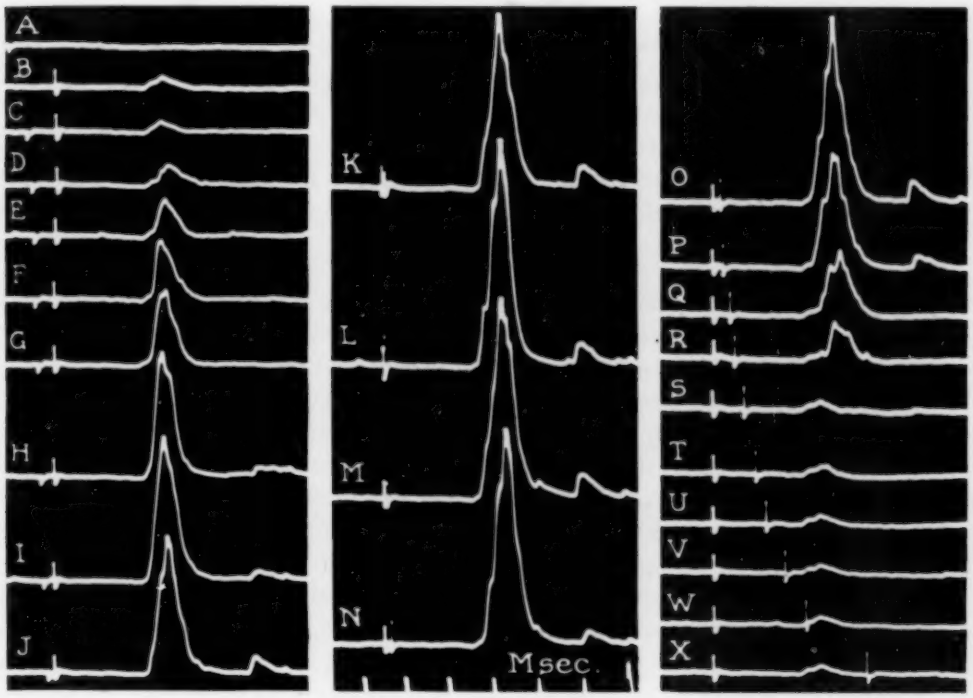

FIG. 6. The summation of peripheral excitations causing the secondary centripetal discharges.

Summation of two excitations by muscle. Figure 6 presents the results of an experiment designed to measure the summation interval of two excitations of nerve at the periphery by muscle action. The L.7 V.R. was split longitudinally so that together with the S.1 V.R. three portions of ventral root were available. Two of these were equipped with stimulating electrodes, through each pair of which a single shock could be delivered. In this way two groups of muscle fibers could be activated independently. The third portion of ventral root was provided with recording leads. By this device the recipient ventral root fibers were entirely freed of primary motor volleys.

Records A and B of Fig. 6 show the results of the two ventral root shocks in isolation. The ventral root volley A is too small to elicit a secondary cen-

tripetal discharge in the recipient root, while the volley B alone evokes a small response. When the volleys A and B are synchronized (record 6L) a large response results, showing that volley A has a subliminal excitatory action on the recipient root endings. In records 6C to 6X the two shocks have been delivered at intervals varying from A 0.7 msec. before B, to B 3.6 msec. before A. Summation of the first discharge group begins in 6D (A −0.6 msec.—B) and ends in 6S (B—0.8 msec.—A). The total summation period for this group then is ca. 1.4 msec.

A second group, arising distal to the first group, first appears in 6H and disappears in 6Q revealing a summation period of 0.9 msec. The second discharge group is absent, in each case, following the shocks in isolation. The full duration of the summation period cannot be determined from the appearance and disappearance of the second group, therefore, since the summated excitation could well be subliminal at each end of the period.

The summation period for excitations of nerve by muscle again suggests the brevity of the causal excitation process.

Summary

When a muscle is activated indirectly by a motor nerve (ventral root) volley, centripetal volleys ensue. These may be recorded in the stimulated ventral root and in neighboring ventral and dorsal roots.

The latency of the secondary centripetal discharges is equal to the sum of conduction time from the stimulating electrodes to the muscle, neuromuscular delay, a short utilization period, and conduction time from the muscle to the recording leads. In the case of secondary centripetal discharges into a stimulated ventral root, the consequences of refractoriness add to the total latency.

The secondary centripetal discharges reflect faithfully the intervention of neuromuscular transmission and muscle action as steps in their causal sequence.

The secondary centripetal discharges are initiated by muscular processes approximately equal in duration to and coincident with the ascending phase of the muscle action potential at or near the junctional region.

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